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THE EFFECT OF ASPHYXIATION OF THE SPINAL CORD ON PAIN SENSIBILITY

A. VAN HARREVELD

From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena

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After application of strychnin (Dusser de Barenne, 1911) or tetanus toxin (Fröhlich and Meyer, 1916) to the spinal cord a marked hyperalgesia of the dermatomes related to the intoxicated segments has been observed. Touching the hyperalgesic parts lightly caused strong defense reactions in the anterior half of the animal. Symptoms of spontaneous pain were also observed.

After recovery of the spinal cord from certain periods of asphyxia, symptoms characteristic of the intoxication with strychnin or tetanus toxin, i.e., high reflex excitability and abolition of reciprocal innervation, have been reported (van Harreveld and Marmont, 1939; van Harreveld, 1939, 1940). The similarity of the effects of strychnin and of asphyxia on reflex excitability made it seem worth while to investigate the effect of asphyxiation of the spinal cord on pain sensibility.

METHOD. The blood circulation in the caudal part of the spinal cord of cats has been prevented for certain periods of time by forcing Ringer's solution, heated to body temperature, into the dural cavity under a pressure higher than the blood pressure (van Harreveld and Marmont, 1939). In previous experiments the area subjected to increased pressure has been limited by ligating dura and cord in the lower thoracic region. Since for an investigation of the sensibility of the hind limbs the spinal cord had to be left intact, another way to block the free passage between the caudal and cranial part of the dural cavity had to be found. As the spinal cord in the lumbar region is wider than the lumen of the vertebral canal in the adjacent thoracic region, it has been found possible to block up the vertebral canal in the lower thoracic region with the lumbar enlargement. After introducing a hypodermic needle into the caudal part of the dural

cavity between the spinous processes of the 6th and 7th lumbar vertebra, the deeply narcotized animal was brought into a maximally flexed position, by which the cord is pulled cranialwards. If in this position Ringer's solution is forced through the needle under a pressure of 22 to 24 cm. of mercury, the lumbar enlargement is pushed into the narrower thoracic part of the vertebral canal, occluding it. The animal can subsequently be brought in a more normal position, since the pressure will keep the lumbar enlargement in place. The pressure has been maintained for periods of 25 to 65 minutes. The reflex excitability and the sensibility of the hind limbs have been examined frequently after asphyxiation of the cord.

This method has been effective in only about one-third of the animals used. In the others the pressure spread throughout the entire dural cavity, stopping the respiration. This is conceivable since this procedure depends on the individually different relative dimensions of the lumbar enlargements and the thoracic spinal canal.

Results. Asphyxiation of the caudal part of the spinal cord affects the walking movements and the spinal reflexes of the hind limbs as well as the sensibility. The changes in the spinal reflexes can be treated briefly, since they were identical with those reported previously, studied in spinal animals (van Harreveld and Marmont, 1939). Data obtained from six groups of animals in which the intact spinal cord had been asphyxiated for respectively 25, 30, 35, 45, 55 and 65 minutes have been collected in table 1.

In 8 cats the cord was asphyxiated for 25 minutes. The first sign of recovery was the development of tone in the extensor muscles or the return of tendon reflexes (knee- and ankle-jerk) 10 to 30 minutes after the end of asphyxia. The flexion reflex returned after an interval of 50 minutes to 5 hours. In five animals the first reaction in the anterior part of the animal to a strong noxious stimulus (pinching of foot and tail) was seen after 50 to 80 minutes. In one animal (25D) it took 8, in two others (25A and E), 24 hours for this reaction to return. Once developed it remained during the entire period of observation.

In all but one of the cats of this group hyperalgesia was observed. In the pronounced cases (indicated in the table with three crosses) the slightest touch of the fur caused a strong defense reaction in the anterior part of the animal. This hyperalgesia was seen most frequently in the foot, sometimes also in the rest of the leg or in the tail. A few times symptoms have been observed which may indicate spontaneous pain as for instance biting of the foot without apparent cause. In other cats (indicated in the table with two crosses) touching or stroking the fur caused little reaction, but a strong defense reaction in the anterior part could be elicited by lightly rolling the toes of the hind limb between the fingers. This stimulus did not cause any reaction when applied to the

TABLE 1

Interval between the end of asphyxia and the first appearance of reflex activity (knee- or ankle-jerk or extensor tone) and of the flexion reflex. First appearance and eventual later disappearance of the first reaction in the anterior part of the animal to a noxious stimulation of hind leg or tail; of hyperalgesia of leg and tail; of hyperalgesia in the area of the 4th lumbar dermatome and of the walking movements. An asterisk indicates that these phenomena remained during the entire period of observation. The group of cats in which the cord was asphyxiated for 25 minutes is indicated as 25A, B, etc., the group of 30 minute cats as 30A, B, etc. The degree of hyperalgesia is indicated with one, two, or three crosses as is explained in the text. The time in this table is given in hours except in those cases in which the number is followed by the letter d, the time is then expressed in days.

DURATION OF ASPHYXIATION	CAT	FIRST REFLEX (KNEE- OR ANKLE-JERK OR TONE)	FLEXION REFLEX	PAIN SENSIBILITY		HYPERALGESIA IN LEG AND TAIL		HYPERALGESIA IN THE DERMATOME L4		WALKING		PERIOD OF SURVIVAL
minutes												
25	A	0.2	5	24	*			48	*	24	*	25d
	B	0.3	1	1	*	48+	*			1.5	*	5d
	C	0.3	1	1.3	*	48++	14d	5d	*	3	48	21d
	D	0.5	2	8	*	30+	*			8	24	5d
	E	0.3	1.8	24	*	48++	*			3	*	23d
	F	0.3	1	1	*	24+	*			1.3	*	7d
	G	0.2	0.9	0.9	*	2+++	*			2.5	*	34d
	H	0.5	0.8	0.8	*	24+++	*			2	48	29d
30	A	0.3	1.8	1.8	*					2	*	4d
	B	0.2	1.8	1.8	*	11+++	*			3	48	15d
	C	0.5	7	2	*							5d
	D	0.8	3.5	2.8	*							4d
	E	0.2	1.5	0.7	*							47d
35	A	0.2	2	1.3	*							5d
	B	0.8	5	1.2	*			48	*	6.5	12	15d
	C	1.5	8.5	2.5	*							46d
	D	0.7	2	3	*	48++	5d			3	48	7d
	E	0.3	3.5	6.5	*			3d	*	1.5	3d	36d
45	A	1.2		24	*							35d
	B	0.8	3.5	1.5	*	2.5+	48	3d	*			6d
	C	1		1.5	*	6.5++	24	4d	10d			23d
55	A	2		3	48							21d
	B	2.5		1.2	*	3.5+++	6					7d
	C	1.4		1.4	*			4d	*			42d
65	A	1.4		1.4	36	2+++	7					15d
	B	1.2		2	10	3++	7					23d
	C	3		1.2	*	1.8+++	5	6d	*			24d

front leg. In a third group of animals (indicated with one cross in the table) only a careful comparison of the reactions to the same stimulus applied to the front and hind legs revealed the hyperalgesia of the posterior limb. This hyperalgesia appeared a long time (24 to 48 hrs.) after asphyxiation in all cases but one, in which it appeared after two hours. It remained usually during the entire period of observation (up to 34 days), though in most cases diminishing in intensity in the course of time. In one cat (25C) hyperalgesia had practically disappeared after 14 days.

In most of the cats with a marked hyperalgesia there existed a hyperexcitability of the flexion reflex, but there seems to be no direct relation between the degree of hyperalgesia and the excitability of the flexion reflex.

All the animals of this group began to make walking movements with the hind limbs 1.3 to 24 hours after the end of asphyxia. But for an exaggerated extensor tone the walking movements were almost normal in most cats a few hours later. In some cases walking remained that way for the period of observation, in others it became notably poorer 24 to 48 hours later, sometimes even disappearing altogether.

In five cats the cord was asphyxiated for 30 and in five for 35 minutes. The interval between the end of asphyxia and the return of tone and tendon reflexes, of the flexion reflex and of the reaction in the anterior part of the cat to strong pinching of foot or tail was not markedly longer than in the 25 minute group of animals. Once developed the reaction to noxious stimuli remained during the whole period of observation.

In two cats (30B and 35D) hyperalgesia was observed beginning 11 and 48 hours after asphyxia. It diminished in the course of time and could not be demonstrated in one cat (35D) 5 days after asphyxia; in the other it remained. In five cats walking movements were observed in the hind limbs some hours after asphyxia, these movements became very poor some hours later and disappeared entirely in four animals after 12 hours to three days.

In three groups of cats each consisting of three animals the spinal cord was asphyxiated for respectively 45, 55 and 65 minutes. In all the animals extensor tone or tendon reflexes returned though after a somewhat longer interval (up to 3 hrs.) than in the 25 to 35 minute groups of cats. This return was usually temporary. The flexion reflex returned (temporarily) in only one cat (45B) in which the cord had been asphyxiated for 45 minutes. The reaction to strong pinching of foot or tail appeared after an interval which was not markedly longer than after the shorter periods of asphyxia. In three cats (55A, 65A and 65B) the return of sensibility was only temporary; it disappeared after 10 to 48 hours. In the other cats the sensibility to noxious stimuli remained during the entire period of observation.

Six of the cats (45B and C, 55B and 65A, B and C) showed hyperalgesia of the hind limbs and tail, beginning 1.8 to 6.5 hours after the end of

asphyxia. This hyperalgesia was temporary and disappeared usually a few hours later. In two cats (45B and C) it remained for respectively 48 and 24 hours. In some of these cats, especially after 65 minutes of asphyxia, the hyperalgesia was the most pronounced ever observed. No walking movements ever returned to these groups of cats.

In eight cats areas of hyperalgesia developed on the medial side of the thigh and on the back cranial to the root of the tail. Usually the hyperalgesia of the thigh was more pronounced. This localized hyperalgesia developed usually later than the hyperalgesia mentioned before, namely, 2 to 6 days after asphyxiation of the cord. It was found more frequently

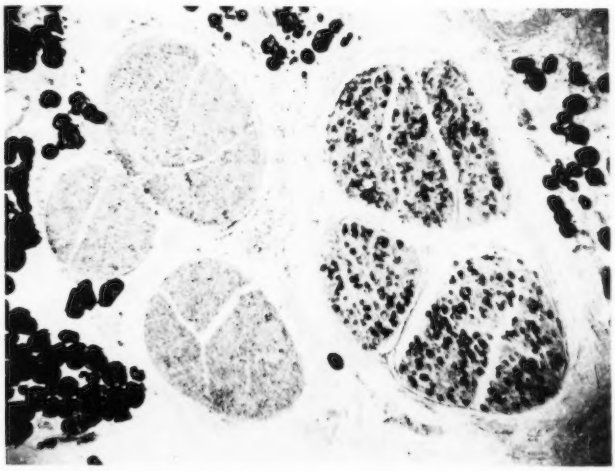

Fig. 1. Dorsal and ventral roots L5 of 55A near the place where the roots penetrate the dura, stained with the Marchi method. The dorsal root (left) does not show any degeneration. An appreciable degeneration is present in the ventral root (right). The large black dots are stained fat cells. Magnification 60 ×.

after the longer periods of asphyxiation, and remained in most cases during the entire period of observation.

Histological examination of the spinal roots. From the cats of which the cord had been asphyxiated for 45 minutes or longer and which had lived afterwards for more than two weeks, the dorsal and ventral roots in the lumbosacral region, stained with the Marchi method, were examined. No degeneration was ever observed in the dorsal roots, whereas a distinct degeneration in the caudal ventral roots was always present (fig. 1). Since even after 65 minutes of asphyxia the dorsal roots did not show degeneration, it can be concluded that asphyxiation had not interfered with the normal sensory supply to the spinal cord.

In two of the five cats thus examined the ventral roots of L5 showed a

strong, in three a moderate degeneration. In the ventral roots of L4 either a slight or no degeneration at all was found. Thus the cranial limit of the area of asphyxiation is situated in the region of the spinal segments L4 and L5.

Discussion. Since the hyperalgesia after asphyxiation remained in many cases until death of the animal (up to 5 weeks after asphyxiation of the cord) this hyperalgesia must be regarded not as a stimulatory effect of asphyxia, but as a release phenomenon, namely, the release from a mechanism which normally prevents impulses set up in the periphery by weak stimuli from reaching the higher centres in such a way that they are experienced as pain. It has to be assumed that this mechanism can be damaged by asphyxia. A similar reasoning with regard to permanent high reflex excitability after asphyxiation of the cord has been given in

TABLE 2

Degeneration in the ventral roots of L6, L5 and L4

Two crosses indicate much, one little degeneration. The animals are marked as in table 1.

DURATION OF ASPHYXIATION	CAT	L6, VENTRAL	L5, VENTRAL	L4, VENTRAL
minutes				
45	A	++	+	−
	C	++	+	+
55	A	++	++	−
65	B	++	+	−
	C	++	++	+

previous papers (van Harreveld and Marmont, 1939; van Harreveld, 1940).

There is a perfect parallel between the development and eventual disappearance of hyperalgesia and that of high extensor tone after asphyxiation of the cord described in a previous paper. Hyperalgesia as well as high extensor tone developed after the shorter periods of asphyxia after a delay of 24 to 48 hours. Once developed they usually remained during the entire period of observation. After the longer periods of asphyxiation both hyperalgesia and high extensor tone returned quicker, but disappeared in a few hours. This course of hyperalgesia and high extensor tone depends presumably, as has been discussed in previous papers, on the greater vulnerability of inhibitory structures and on the ability of neurons, damaged so severely that they will be destroyed shortly, to resume their function temporarily. The latter peculiarity is demonstrated in the present experiments by the walking movements which in many cases returned temporarily after asphyxia. It has to be assumed that after the shorter periods of asphyxia both the structures for the conduction

of the hyperalgetic pain sensation and the structures normally preventing hyperalgesia by blocking this path resume their function; the former permanently, the latter, being more sensitive to asphyxia, temporarily. After a certain time the structure blocking the path used during hyperalgesia stops functioning and a permanent hyperalgesia develops.

After the longer periods of asphyxia the structures normally blocking the path of pain in hyperalgesia are damaged so severely that they will hardly recover at all, whereas the path of the pain sensation recovers temporarily, resulting in a temporary hyperalgesia. Thus the disappearance of hyperalgesia in the latter cases means that the path of the impulses used in hyperalgesia is destroyed and if in these animals pain still can be elicited it has to take another path. In all the cats in which the cord had been asphyxiated for longer periods of time pain reactions could be elicited by strong noxious stimuli after hyperalgesia had disappeared. Thus we have to assume that there are two systems which can conduct impulses resulting in pain sensations. One is normally not apparent, but can under certain conditions (as asphyxiation of the cord) become highly excitable; it is stimulated by stimuli which are not noxious. The second system is stimulated by noxious stimuli. It is more resistant to asphyxia since it was abolished, after returning temporarily, only after the longest periods of asphyxia.

The hyperalgesia localized on the medial side of the thigh and on the back cranial to the root of the tail can be explained in the following way. After the longer periods of asphyxia the cord has been damaged too severely to show a permanent hyperalgesia but at the limits of the area of increased pressure where the asphyxiated cord changes into the normal there must be a region damaged to the right degree to cause this. If this area is wide enough we will observe a permanent hyperalgesia in the related dermatome. According to de Boer's determination of the dermatomes of the cat (1929) both these hyperalgesic spots are part of the dermatome L4 and it has to be assumed that in the spinal segment L4 the circumstances are favorable for the development of this kind of hyperalgesia. The histological examination of the roots showed in agreement with this that the limit of the asphyxiated area is situated in this region.

The hyperalgesia after asphyxiation of the cord is probably not related to the hyperalgesia observed by many authors (see Foerster, 1927) after the severance of the dorsal columns. Foerster has compared the latter form of hyperalgesia with the hyperalgesia after abolition of the epicritic sensibility, which has been explained by Head (1893, 1894, 1896) as a lack of inhibition of the protopathic by the epicritic sensibility. Foerster implies that in transecting the dorsal columns the fibers for the epicritic sensibility are transected. Since these fibers are a direct continuation of the fibres of the dorsal roots and since in the present experiments the latter

were always found to be absolutely intact, there is no reason to believe that their spinal continuation has been damaged by asphyxia.

Fabricius (cited from Foerster, 1927) assumed the existence of a centrifugal tract in the lateral columns which normally inhibits pain sensations. It could be assumed that asphyxia damages spinal structures related to this tract. In Fabricius' conception pain sensations are inhibited by impulses arising in supraspinal structures. It is quite possible, however, that the structure normally preventing hyperalgesia is a strictly spinal one. The existence of a spinal mechanism normally depressing reflex activity (van Harreveld and Marmont, 1939; van Harreveld, 1940) is in favor of this. The assumption of a strictly spinal mechanism readily explains cases of hyperalgesia in which changes in a spinal segment or in the structures related to it result in hyperalgesia in that segment only. Several instances of this kind of hyperalgesia can be given; the hyperalgesia in a dermatome after treatment of the related spinal segment with strychnin, the referred pain and hyperalgesia in dermatomes related to the spinal segments which are connected with a diseased internal organ (Head, 1905), the hyperalgesia after the abolition of the epicritic sensibility (Head, 1893, 1894, 1896). In all these cases hyperalgesia may be caused by influencing the spinal structure which in the normal animal prevents hyperalgesia and which in the present experiments has been damaged by asphyxiation of the cord.

In true neuralgias little significant changes have been found in the first sensory neurone. This is one of the reasons why Frazier, Lewy and Rowe (1937) tried to localize the pathological process underlying neuralgias in the thalamus. The hyperalgesia after asphyxiation of the cord suggests, however, that processes located in the neuraxis may be the cause of this condition.

SUMMARY

Hyperalgesia has been observed in the hind limbs of cats after asphyxiation of the caudal part of the spinal cord. After the shorter periods of asphyxiation (25 min.) this hyperalgesia usually remained for the entire period of observation (up to 5 wks.). After the longer periods of asphyxiation the hyperalgesia was temporary. In the not hyperalgesic parts of the hind limbs or after the disappearance of hyperalgesia in cases in which it occurred temporarily the leg was sensitive to strong noxious stimuli. The long duration of hyperalgesia observed in many cases leads to the conclusion that hyperalgesia is a release phenomenon. The similarity of the course of hyperalgesia and that of high extensor tone after asphyxiation of the cord suggests that both will have to be explained in the same way, namely, by the assumption that asphyxiation damages structures in the cord which normally prevent hyperalgesia or depress extensor tone.

Reasons are presented for the opinion that different structures are involved in the transport of the impulses for pain during hyperalgesia and for the impulses elicited by strong noxious stimuli.

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HEPATIC ACETONE BODY PRODUCTION IN THE DOG DURING FASTING AND FAT FEEDING

LATHAN A. CRANDALL, JR., H. BERRY IVY AND GEORGE J. EHNI

From the Department of Physiology, University of Tennessee College of Medicine, and the Department of Physiology and Pharmacology, Northwestern University Medical School

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Most of the evidence concerning the production of acetone bodies by the liver has been obtained by indirect methods or from observations on perfused organs or tissue slices. It was believed that data of value might be obtained from analyses of the blood entering and leaving the liver, since ketogenesis appears to be a function of this organ only. We have therefore employed the angiostomy technique, uncomplicated by anesthesia, for a study of hepatic acetone body production, and have chosen fasting or fat feeding as the most nearly "normal" stimulus for ketogenesis.

Our observations have led us to the conclusion that hepatic ketogenesis is an alternative pathway for fat oxidation that is brought into play only when the supply of carbohydrate is inadequate. Unpublished data (Ivy and Crandall) indicate that during fasting there are qualitative changes in the metabolism of lactic acid also. We suggest that such metabolic processes, occurring under conditions of carbohydrate lack and differing qualitatively from those of non-fasted animals, may be referred to as phases of the special metabolism of glucoprivia.

METHODS. Normal angiostomized dogs (London cannulae on portal and hepatic veins) were used; the sources of error in this technique have been discussed previously (1). Total blood acetone bodies were determined according to the method of Crandall (2), which is believed to be accurate within 0.1 mgm. per 100 ml. for the low values found in non-fasting animals. All values are expressed as β-hydroxybutyric acid. Determinations of acetone body and glucose concentrations in hepatic, portal, and arterial blood samples have been made in 15 instances on 11 dogs within 24 hours after the last meal and in 25 experiments on 8 dogs that had fasted 2 to 11 days. After fasting, some of the animals were given 100 ml. of olive oil by mouth each day for periods up to 9 days, 13 observations were made on 9 such animals. At the end of the periods of fasting or fat feeding some dogs were given 20 grams of glucose by stomach tube, the hepatic acetone body and glucose outputs being determined be-

fore and at intervals up to 1½ hours after the glucose administration; 6 such experiments on 4 dogs are reported.

The 24 hour output of acetone bodies by the liver was calculated on the assumption that the portal vein contributed ¾ and the hepatic artery ¼ of the blood reaching the liver; that the concentrations of acetone bodies found in the portal, hepatic, and arterial samples were averages for the day; and that the rate of blood flow through the liver was 28.6 ml. per kilo per minute, which is the average found by Blalock and Mason (3). Because of unknown variations from these assumed averages, the amounts of acetone bodies estimated to be formed by the liver for the 24 hour periods are only rough approximations, and are to be regarded as indicating only the order of magnitude of hepatic ketogenesis. It may be noted, however, that variations in the proportions of blood supplied by the portal vein and hepatic artery would have little effect since the amounts removed by the intestinal tract are small.

The concentration of acetone bodies in the urine was determined by the method of Crandall (2) after removal of interfering substances by precipitation with copper sulfate and calcium hydroxide.

Statistical methods have been applied wherever possible.

Results. Data for animals fed 18 to 24 hours prior to determinations of acetone body concentrations in hepatic, portal, and arterial blood will not be presented individually, since there was little variation from one dog to another. The arterial blood acetone body levels varied from 0.5 to 1.4 mgm. per 100 ml. In no instance did the concentrations in the three samples differ by more than 0.2 mgm., which is regarded as within the limits of error of the method. The mean change produced by the liver in our 15 observations was a retention of 0.01 mgm./100 ml., with a standard error of 0.03. It is apparent that the liver is producing no change in blood acetone bodies that is detectable by the method employed, and that the maximum hepatic output that could have escaped detection is 0.1 mgm./100 ml., which is insignificant.

The data on the fasted (table 1) and fat fed (table 2) animals provide a picture of the development and intensity of ketosis in the dog. It begins gradually after 2 to 4 days of fasting. After 5 to 11 days the liver is adding 1.5 to 8.1 mgm. of ketone bodies to each 100 ml. of blood. The average rate of ketone body liberation for each dog in table 1 on the last day of fasting is 3.1 mgm. per 100 ml., with a standard error of ± 0.51. The rate for the dogs that had been fasted and then fed fat for three or more days is 6.3 ± 1.11. The difference between the rates of these two groups is less than three times its standard error, and would be of questionable significance in any case since the effect could be attributed to longer lack of carbohydrate as well as to the fat feeding. If fat feeding does intensify ketosis, which seems probable, the effect is not great.

The utilization of acetone bodies by those tissues drained by the portal vein can be estimated from the data in tables 1 and 2. On the last day of fasting (table 1) it averaged 0.84 mgm. per 100 ml. For the fat fed animals (table 2) it amounted to 1.2 mgm. per 100 ml. (S.E. ± 0.25).

As might be anticipated, there is a positive correlation between rate of hepatic ketogenesis in milligrams per 100 ml. of blood, and the concentra-

TABLE 1

Hepatic, portal and arterial blood acetone bodies (expressed as milligrams beta-hydroxybutyric acid per 100 ml. of blood) at various intervals during starvation

The urinary acetone body excretion for the corresponding 24 hours is compared with the calculated 24 hour hepatic acetone body formation

DOG	DAYS FASTED	HEPATIC	PORTAL	ART.	HEPATIC OUTPUT	HEPATIC OUTPUT PER 24 HOURS (CALCULATED)	24 HOUR URINARY OUTPUT
					mgm./100 ml.	*grams*	*grams*
1	3	3.3	2.9	3.0	0.4	1.7	
1	8	7.6	3.8	5.0	3.5	15.0	0.238
2	3	3.1	2.6	2.4	0.5	1.9	
2	5	6.3	2.2	4.0	3.6	13.4	
3	3	1.1	1.0	1.0	0.1		
3	6	3.6	1.9	2.5	1.5	8.2	
4	3	2.6	2.1	1.9	0.5	1.8	0.039
4	7	2.8	1.1	1.7	1.6	5.6	
5	6	3.6	1.9	1.9	1.7	9.3	0.089
6	2	0.8	0.8	0.8	0.0		
6	3	2.2	0.9	1.0	1.3	9.4	0.024
6	4	2.0	0.5	1.0	1.4	10.0	0.031
6	6	2.3	1.4	1.7	0.8	5.6	0.090
6	11	8.5	4.1	4.7	4.2	27.4	0.541
7	2	2.5	1.7	2.2	0.7	5.3	0.088
7	3	7.8	2.8	4.3	4.6	34.3	0.341
7	4	10.5	2.8	4.5	7.3	54.2	0.368
7	6	10.6	4.1	6.1	6.0	43.1	0.134
7	11	12.9	7.1	8.1	5.5	37.6	0.239
8	2	1.8	1.6	1.6	0.2		
8	4	2.3	0.9	1.1	1.3	7.1	0.060
8	6	5.7	2.6	3.8	2.8	15.1	0.158
8	9	5.6	2.5	3.4	2.9	15.4	0.105

tion of ketone bodies in the blood. The correlation coefficient (r) for the fasted animals (table 1) is 0.86 ± 0.21; for the whole series of fat fed and fasted dogs r is 0.58 ± 0.17. The fact that these coefficients are significant may be taken as evidence that determination of hepatic output in terms of mgm. per 100 ml. of blood flow indicates the order of magnitude of the hepatic output per unit of time, i.e., that blood flow variations are not

sufficiently great to impair seriously the value of calculated daily ketone body formation since it is obvious that rate of ketogenesis and rate of utilization determine blood concentration. The amount of ketone bodies formed in 24 hours, according to such calculations, averages 21.6 grams for dogs fasted 4 or more days, and 32.1 grams for those fasted and then fat fed for 3 or more days. The urinary acetone body output for the fasted animals averages 0.187 gram and for the fat fed dogs 0.482 gram. The urinary output is therefore 0.6 per cent of the calculated hepatic production in the fasted and 1.5 per cent in the fat fed dogs. Even though the calculated hepatic output were twice the actual production, the amount of ketone bodies eliminated in the urine would still be an insignificant fraction of the amount formed. One can come to no other conclusion but that at least 95 per cent of the acetone bodies must be oxidized by the tissues. This is in accordance with previous evidence (4, 5).

TABLE 2

Hepatic, portal, and arterial acetone bodies (expressed as milligrams beta-hydroxybutyric acid per 100 ml. of blood) in dogs that have been fasted and then fed 100 ml. of olive or peanut oil per day for several days

The urinary acetone body output per 24 hours is compared with the calculated hepatic acetone body formation for the same 24 hour period

DOG	DAYS FASTED	DAYS FAT FED	HEPATIC	PORTAL	ARTERIAL	HEPATIC OUTPUT	HEPATIC 24 HOUR OUTPUT (CALCULATED)	24 HOUR URINARY OUTPUT
							grams	*grams*
1	8	3	7.5	2.9	5.8	3.9	16.6	0.442
1	8	6	12.0	8.1	10.2	3.4	14.3	0.510
2	5	1	5.6	3.2	4.1	2.2	7.6	
2	5	5	15.2	7.3	8.4	7.5	27.0	0.346
4	7	6	14.4	10.5	12.2	3.5	11.9	0.420
5	6	6	9.6	6.3	7.4	2.7	14.3	0.170
6	11	6	27.0	10.9	11.6	15.9	100.1	0.798
7	11	7	15.3	10.0	9.6	5.4	36.7	0.389
8	9	1	5.7	2.8	3.1	2.8	14.8	0.221
8	9	3	14.8	9.8	10.2	4.9	25.7	0.288
9	6	7	18.6	11.7	12.6	6.8	35.4	0.912
9	6	9	46.8	37.7	40.0	8.5	43.0	0.440
10	6	9	37.4	30.6	31.4	6.6	27.8	0.591

The oral administration of glucose promptly reduces ketogenesis in the liver, as shown in table 3. The rate of decrease in arterial acetone body concentration in the last four experiments varies from 0.20 to 0.26 mgm. per 100 ml. of blood per minute; it cannot be estimated for the first two experiments since the arterial concentration had reached normal levels before the first post-glucose samples were taken. This provides evidence

TABLE 3

The effect of the oral administration of 20 grams of glucose on the hepatic acetone body production of dogs made ketotic by fasting or by fasting followed by daily fat feeding

All results expressed as milligrams beta-hydroxybutyric acid per 100 ml. of blood

DOG	KETOSIS PRODUCED BY	MINUTES AFTER GLUCOSE	ARTERIAL ACETONE BODY CONCENTRATION	HEPATIC ACETONE BODY OUTPUT
6	8 day fast	Before	3.1	3.6
		30	0.7	0.0
		90	0.3	0.2
7	8 day fast	Before	5.8	5.4
		30	0.7	0.3
		90	0.5	0.0
6	11 day fast, then 6 days fat feeding	Before	11.6	15.9
		45	2.5	0.7
		90	1.0	0.0
7	11 day fast, then 7 days fat feeding	Before	9.6	5.4
		30	2.9	1.7
		60	2.5	1.0
8	9 day fast, then 4 days fat feeding	Before	10.2	4.9
		15	6.1	3.4
		30	2.5	2.3
9	6 day fast, then 11 days fat feeding	Before	27.2	6.9
		30	19.4	5.0
		60	13.3	3.5

TABLE 4

Mean hepatic output of glucose and of glucose plus acetone bodies and standard errors in non-fasting and fasting dogs

Data from previous communication included for comparison with those from present series

NUMBER OF EXPERIMENTS	SOURCE OF DATA	DAYS FASTED	HEPATIC GLUCOSE OUTPUT	HEPATIC OUTPUT GLUCOSE PLUS ACETONE BODIES
47	Cherry and Crandall, This Journal **125:** 41, 1939	Less than 1	9.1 ±0.68	
20	All dogs, this series	Less than 1	9.2 ±0.57	
28	All dogs, this series	More than 3	4.7 ±0.41	9.4 ±0.63

for the antiketogenic effect of glucose, but does not disprove the possibility that glucose may also be ketolytic.

Table 4 presents the mean values, with their standard errors, for hepatic

output of glucose and of glucose plus acetone bodies in the present series of animals. For comparison the mean glucose output for a larger series as given by Cherry and Crandall (6) is also shown. Because some of the observations were made in Memphis and others in Chicago, the data obtained in the two locations were compared so that any differences in hepatic glucose production due to different laboratory conditions would be revealed. No such differences were found. It is evident that fasting for more than 3 days reduces the amount of glucose liberated by the liver per unit volume of blood almost 50 per cent. When the hepatic acetone body output for each dog is added to its output of glucose, the mean value of the totals is found to be in the same range as the non-fasting output of glucose alone. That is, the statistically significant decrease in hepatic output on fasting is compensated by acetone body production.

Discussion. One of the most salient observations we have made is that the liver does not add acetone bodies to the blood before the second day of fasting in the dog; i.e., ketogenesis does not occur in the normal animal in the post-absorptive state. Since there is good evidence that the liver is the sole site of acetone body formation (7, 8), the absence of ketogenesis correlates well with the low concentration of acetone bodies in the blood of the non-fasting animal. A number of investigators (9, 10, 11, 12, 13) appear to believe that acetone body formation occurs in the non-fasting animal, others have suggested (8, 14, 15, 16) that it is dependent on a decrease in liver glycogen to a critical level. The evidence presented here is believed to be the first direct proof that ketogenesis does not occur when glucose oxidation is not impaired by carbohydrate lack or by some metabolic abnormality.

That fat is oxidized to an appreciable extent in the non-fasting animal can hardly be denied. The lack of formation of acetone bodies under conditions in which fat is being oxidized demonstrates the existence of two alternative routes for fat utilization, one through the ketone bodies and a second which remains unknown but which does not involve ketone body production. Teleologically speaking, the existence of the alternative route which involves acetone body formation argues that when the glucose supply is inadequate the tissues need this type of compound, which is more analogous to glucose than to fat in its proportions of carbon, hydrogen and oxygen.

From the values for daily acetone body output by the liver given in tables 1 and 2 it can be estimated that hepatic ketogenesis can supply up to 57 per cent of the total caloric requirement of the body, and that in the fat fed dogs this process accounts, on the average, for 17 per cent of the total heat production. Such calculations are subject to considerable error and their chief value is to indicate that an appreciable fraction of the total metabolism may be due to acetone body oxidation.

If the hepatic blood flow during fasting does not differ substantially from that of the non-fasting dog, the rate of hepatic glucose production decreases by almost 50 per cent after the third day of starvation. This may explain the decreased blood sugar levels that have been found during fasting in various species (17, 18, 19). We have also observed that the blood sugar concentrations in our fasted dogs may be below what would be considered normal for these animals in the post-absorptive state.

It is of especial interest that the hepatic energy output of the liver of the fasting dog (as glucose plus ketone bodies) is equivalent to its energy output in the non-fasting state (as glucose only.) This again suggests that it it may be essential for the extra-hepatic tissues to receive a certain definite proportion of their fuel in a partially oxidized form.

The following hypothesis is offered in explanation of the occurrence of ketosis: 1, the extra-hepatic tissues require that a certain proportion of their metabolic requirement (presumably varying with the tissue) be supplied by glucose or by acetone bodies; 2, acetone body production therefore decreases the need for glucose production by the liver and conserves the store of carbohydrate and glucose precursors; 3, during fasting or fat feeding the liver maintains a relatively constant output of energy in the form of glucose or glucose plus ketone bodies, the mechanism involved being unknown but the primary stimulus for acetone body production being a lack of preformed carbohydrate and/or an inability to carry out gluconeogenesis at a rate that will meet the total requirement.

Since the completion of these studies, Barnes *et al.* (12) have reported observations on acetone body utilization in the leg of the intact animal. It should be noted that their data are in every respect compatible with the hypothesis here outlined.

We have come to the conclusion that ketogenesis is a special process, in that it does not occur when food is allowed *ad libitum*, and that it appears in response to a failure of the carbohydrate supply caused by fasting or high fat diets.

There is no reason, apparent to us, for regarding ketosis as an abnormal condition except insofar as fasting may be so regarded. Rather it should be looked upon as a mechanism by which the body meets a condition of stress which is likely to occur in the everyday life of most individuals of whatever species.

SUMMARY

Experiments on unanesthetized angiostomized dogs have provided the following information:

1. After 2 to 4 days of fasting the liver begins adding acetone bodies to the blood. Prior to this time no hepatic acetone body production is detectable.

2. The urinary excretion of acetone bodies in our experiments amounts to only a small fraction (probably not more than 5 per cent) of the total amount formed.

3. Assuming that fasting does not produce large changes in the rate of hepatic blood flow, it can be calculated that: *a*, oxidation of acetone bodies may supply up to 50 per cent of the total caloric requirement of the dog after the fifth day of fasting; *b*, after 3 or more days of fasting the hepatic glucose output drops approximately 50 per cent below that observed in the post-absorptive state; *c*, the output of glucose plus acetone bodies during fasting in terms of milligrams or of energy available from their oxidation is equivalent within the limits of error of our determinations to the output of glucose alone in the fasted dog prior to the onset of ketosis.

5. Glucose administered orally is antiketogenic; our data do not permit conclusions concerning a possible ketolytic effect of glucose.

On the basis of these observations it is suggested that there are two alternative pathways for fat oxidation, one through acetone body production in the liver and an alternative pathway not involving ketogenesis. The hypothesis that ketogenesis is a mechanism for supplying the tissues with substances which can be used to replace partially the rôle of glucose in metabolism, and therefore to conserve the carbohydrate reserve and/or to decrease the need for gluconeogenesis, is offered.

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OBSERVATIONS UPON THE PRESSOR SUBSTANCE CAUSING THE RISE IN BLOOD PRESSURE FOLLOWING THE TERMINATION OF TEMPORARY, COMPLETE RENAL ISCHEMIA

SIDNEY D. LEO, MYRON PRINZMETAL AND HARVEY A. LEWIS

From the Departments of Physiology and Medicine, University of Southern California School of Medicine[1]

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In 1938 Taquini (1), modifying the Goldblatt procedure in dogs, found that reëstablishment of the circulation of kidneys rendered completely ischemic for several hours resulted in a rise in blood pressure. This observation has been confirmed in dogs and extended to cats, rats and guinea pigs (2), although negative results were obtained in rabbits (3).

The hypertension which is due to chronic, partial renal ischemia (Goldblatt) has been the subject of extensive investigation, but few studies have been reported concerning the nature of the rise in blood pressure which occurs upon the termination of temporary, complete renal ischemia (Taquini). It is possible that the factors underlying both types of hypertension are closely related or identical. Certain observations suggest that in chronic, partial renal ischemia the kidney manufactures a pressor substance which is continuously discharged into the general circulation in relatively small amounts, causing chronic hypertension. It may be similarly supposed that in temporary, complete renal ischemia, the same or a related pressor substance accumulates in the kidney and upon restoration of the renal circulation is released suddenly into the blood stream in relatively large amounts, giving rise to acute hypertension.

In 1898 Tigerstedt and Bergmann (4) reported that extracts of normal kidneys contain a pressor substance, which they named renin. It has since been suggested that this substance may be the pressor principle which is responsible for the hypertension resulting from renal ischemia. In 1936 Harrison, Blalock and Mason (5) and Prinzmetal and Friedman (6), working independently, found that the amount of renin in extracts of kidneys of dogs with Goldblatt hypertension is greater than that in control normal kidneys of the same animals.

It is the purpose of this paper to report the following investigations on the nature of the pressor substance which appears to be responsible for the pressor reaction resulting from the termination of complete renal ischemia:

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1. The effect of preliminary injection of piperidomethyl-3-benzodioxane (933F) upon the expected rise in blood pressure resulting from the termination of complete renal ischemia.

2. The effect of preliminary induction of tachyphylaxis to renin upon the expected rise in blood pressure which results from the termination of complete renal ischemia.

3. The comparative pressor effects of extracts of the completely ischemic and control normal kidney of the same animal.

Experiment I—The effect of 933F on the rise in blood pressure following the reëstablishment of circulation in the completely ischemic kidney. 933F is one of many similar compounds synthesized by Fourneau which possess the property of abolishing or reversing the effects of epinephrine injection. 933F has been found to be of distinct value in the study of the nature of pressor substances because of its selective action (7–14), for in doses which reverse the pressor effect of epinephrine and epinephrine-like compounds almost all other epinephrine responses remain unchanged. Katz and Friedberg (15) found that the preliminary injection of 933F in normal and hypertensive dogs abolished or reversed the pressor reaction to epinephrine but did not prevent the pressor effect of renin. These authors concluded that renin is not sympathomimetic in action.

The following experiments were devised to study the effect of 933F on the acute hypertension following the termination of temporary, complete renal ischemia. If this post-ischemic pressor effect is of an epinephrine-like nature, an injection of 933F prior to the restoration of the renal circulation should prevent the rise in blood pressure in the same manner as it does in the instance of epinephrine. The effect of 933F upon the pressor reaction to renin was also investigated.

Method. Each of ten cats was anesthetized with ether, the abdomen opened and one renal pedicle completely occluded by means of a bulldog clamp. The abdomen was then closed and the animal allowed to recover from the anesthetic. Four to six hours later the animal was reanesthetized with nembutal intraperitoneally, the abdomen reopened through the previous incision and the clamp exposed. The carotid artery was cannulated and the blood pressure recorded in the usual manner. Epinephrine (0.025 mgm.) was injected intravenously. After the blood pressure had returned to the pre-injection level, 933F in dosage of 1.5–3 mgm. per kilogram was similarly administered. The blood pressure was again allowed to return to its original level and the previous injection of epinephrine repeated. The clamp occluding the renal pedicle was then released after a constant level in blood pressure had been established. When the blood pressure had again returned to a constant level following the release of the clamp, 0.025 mgm. of epinephrine was injected, this time into the substance of the kidney. In four instances additional injections of 1 cc. of renin (prepared from the kidneys of cats by the method of Pickering and Prinzmetal (16)) were made into the external jugular vein before and after the injection of 933F. In a fifth animal an injection of 1 cc. of renin was given only after the administration of 933F.

RESULTS. Preliminary injection of epinephrine caused a characteristic pressor response in each instance. Following the injection of 933F the pressor effect of epinephrine was reversed. Removal of the clamp occluding the renal pedicle was followed by a rise in blood pressure in all but one instance, the average rise in blood pressure for all ten animals being 30 mm. of Hg. The injection of epinephrine into the substance of the kidney after the pedicle had been released resulted in a lowering of the blood pressure in each instance, the average fall being 36 mm. of Hg. This proved that the circulation to the kidney had been reëstablished and that the effect of 933F was still operative. The injection of renin, both before and after the administration of 933F, caused an elevation in blood pressure

TABLE 1

The effect of 933F on the pressor response following reëstablishment of the circulation of completely ischemic kidneys of cats

ANIMAL NUMBER	BLOOD PRESSURE RESPONSE TO 0.025 MGM. EPINEPHRINE	BLOOD PRESSURE RESPONSE TO RENIN	BLOOD PRESSURE RESPONSE TO SECOND EPINEPHRINE	BLOOD PRESSURE RESPONSE TO SECOND RENIN	BLOOD PRESSURE RESPONSE TO REËSTABLISHMENT OF CIRCULATION OF ISCHEMIC KIDNEY
1	+50		−30		−10
2	+26		−40		+6
3	+44	+18	−54	+14	+12
4	+56	+16	−60	+34	+12
5	+60	+26	−60	+24	+104
6	+30		−72		+4
7	+50		−34		+42
8	+60	+40	−40	+46	+20
9	+64		−38	+20	+16
10	+100		−48		+52
Averages...	+54	+25	−47	+26	+30

in all animals so treated; the average rise before 933F, in four animals, was 25 mm. of Hg, and the average rise after 933F, in five animals, was 26 mm. of Hg (fig. 1, table 1).

Experiment II—The effect on the blood pressure of reëstablishment of the circulation in the completely ischemic kidney of animals rendered tachyphylactic to renin. Tigerstedt and Bergmann (4) found that repeated injections of renin resulted in progressively diminishing pressor responses, a phenomenon known as tachyphylaxis. The nature of this phenomenon has not been fully elucidated, although Page and Helmer (17) believe that renin-activator, a substance present in normal blood and necessary for the activation of renin, may be depleted by repeated injections of renin, thus causing diminishing responses.

The following experiment was devised to determine whether or not the

hypertension which results from the reëstablishment of the circulation in the completely ischemic kidney is prevented in animals rendered tachyphylactic to renin. A significant reduction or abolition of the pressor response would suggest that the post-ischemic hypertension is due to renin or a renin-like substance.

Method. Twenty-two cats were anesthetized with ether. In fourteen animals one renal pedicle was completely occluded by means of a bulldog clamp. In the remaining eight, both renal pedicles were similarly occluded. Four to six hours later each animal was reanesthetized with nembutal intraperitoneally, and the blood pressure recorded as before. The abdomen was then reopened and the clamp or clamps exposed. In eleven of the twenty-two animals, repeated injections of renin

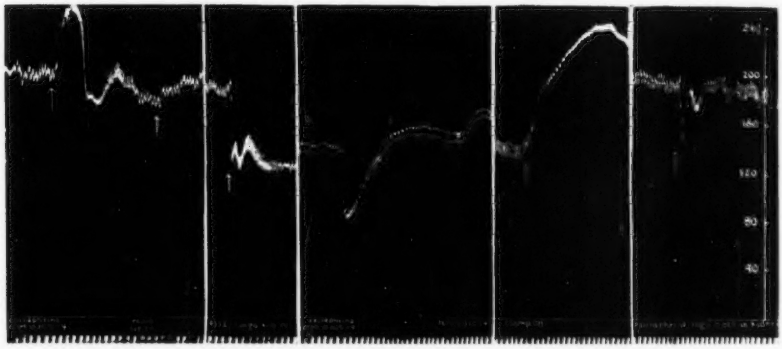

Fig. 1. Effect of previously administered 933F on the expected rise in blood pressure following the reëstablishment of circulation in the ischemic kidney of the cat. At first arrow 0.025 mgm. of epinephrine i. v. At second arrow 1 cc. of renin i. v. At third arrow i. v. 933F in dosage of 3 mgm. per kilogram. At fourth arrow 0.025 mgm. of epinephrine i. v. At fifth arrow 1 cc. of renin i. v. At sixth arrow release of clamp completely occluding renal pedicle for five hours. At seventh arrow 0.025 mgm. of epinephrine injected into substance of kidney with reëstablished circulation. Blood pressure in mm. Hg. Time marker 15 seconds.

were administered in the external jugular vein. Except in one instance, in which purified renin prepared by the method of Helmer and Page (18) was used, the renin injected was prepared from the kidneys of cats by the method of Pickering and Prinzmetal (16). It required five to twenty-one injections for the induction of tachyphylaxis. At this point, when a constant level in blood pressure had been established, the clamp (3 animals) or clamps (8 animals) occluding the renal pedicle or pedicles were released and changes in blood pressure observed. In the eleven remaining animals, which served as controls, only one pedicle was clamped. In these no renin was injected, and the occluding clamp was released as soon as a constant level in blood pressure was obtained.

Results. In eight of the eleven experiments in which induction of tachyphylaxis preceded restoration of the circulation in the ischemic kidney, no rise in blood pressure followed the removal of the clamp or clamps

occluding the renal pedicles; small rises in blood pressure (8, 10 and 10 mm. of Hg, respectively) were observed in the three remaining animals. The

TABLE 2A

Effect of reëstablishment of circulation of completely ischemic kidneys of cats following induction of tachyphylaxis by repeated injections of renin

ANIMAL NUMBER	NUMBER OF INJECTIONS OF RENIN	CIRCULATION REËSTABLISHED IN 1 OR 2 KIDNEYS	BLOOD PRESSURE BEFORE RE-ESTABLISHMENT OF CIRCULATION	BLOOD PRESSURE FOLLOWING RE-ESTABLISHMENT OF CIRCULATION	NET RISE IN BLOOD PRESSURE
			mm. Hg	*mm.* Hg	*mm.* Hg
1	6	1	214	214	0
2	21	1	170	170	0
3	11	1	176	186	10
4	16	2	222	222	0
5	5	2	168	168	0
6	13	2	188	188	0
7	9	2	144	154	10
8	11	2	206	206	0
9	12	2	190	190	0
10	12	2	198	206	8
11	9	2	206	206	0
Averages..	11		189	191.5	2.5

TABLE 2B

Effect of reëstablishment of circulation of completely ischemic kidneys of cats

ANIMAL NUMBER	CIRCULATION REËSTABLISHED IN 1 OR 2 KIDNEYS	BLOOD PRESSURE BEFORE RE-ESTABLISHMENT OF CIRCULATION	BLOOD PRESSURE FOLLOWING RE-ESTABLISHMENT OF CIRCULATION	NET RISE IN BLOOD PRESSURE
		mm. Hg	*mm.* Hg	*mm.* Hg
1	1	144	172	48
2	1	127	182	55
3	1	162	242	80
4	1	128	168	40
5	1	144	184	40
6	1	140	176	30
7	1	143	170	27
8	1	125	148	23
9	1	166	186	20
10	1	132	156	24
11	1	160	188	28
Averages.....		143	181	39

average rise in blood pressure in these eleven tachyphylactic animals was 2.5 mm. of Hg (fig. 2a, table 2a). In the eleven control animals a significant elevation in blood pressure was noted in each instance upon release of

the clamp occluding the renal pedicle, the average rise being 39 mm. of Hg (fig. 2b, table 2b).

Experiment III—Comparison of the pressor effects of extracts of completely ischemic kidneys and control normal kidneys of the same animals. It has been found that the amount of renin is greater in partially ischemic kidneys of hypertensive dogs than in control normal kidneys of the same

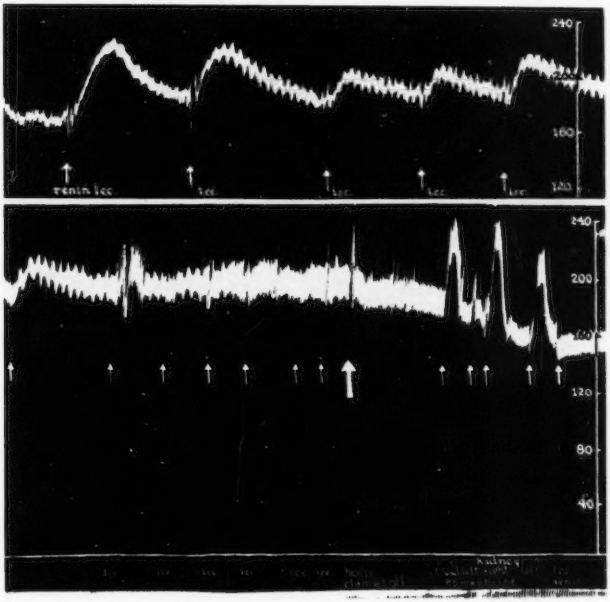

Fig. 2a. Effect on blood pressure of reëstablishment of circulation in both ischemic kidneys of a cat previously rendered tachyphylactic to renin. First twelve small arrows indicate intravenous injections of 1 cc. of renin. Clamps completely occluding both renal pedicles for five hours released at large arrow. Following four small arrows indicate injections of 0.025 mgm. of epinephrine, first intravenously, then into substance of left, right, and again left kidney; 1 cc. of renin injected intravenously at last small arrow. Blood pressure in mm. Hg. Time marker 15 seconds.

animals (5, 6). It is therefore important to compare the amount of renin in kidneys rendered completely ischemic with that in control normal kidneys of the same animals. For this reason the following experiment was performed:

Method. Ether anesthesia was employed in each of eleven cats. One renal pedicle was occluded. Four to six hours later the cat was re-anesthetized with nembutal intraperitoneally, and both the normal and ischemic kidneys removed.

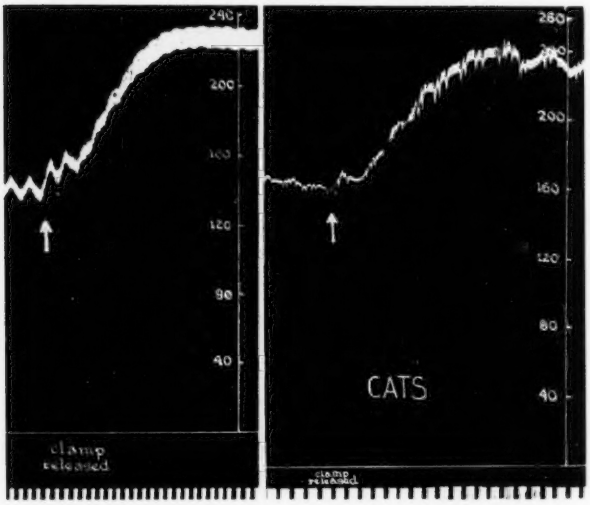

Fig. 2b. Effect on blood pressure of reëstablishment of circulation in one ischemic kidney of a non-tachyphylactic cat. Clamp completely occluding renal pedicle for five hours released at arrow. Blood pressure in mm. Hg. Time marker 15 seconds.

TABLE 3

Assay of renin in completely ischemic and control normal kidneys of cats

ANIMAL NUMBER	PRESSOR EFFECT OF RENIN EXTRACTED FROM NORMAL KIDNEY	PRESSOR EFFECT OF RENIN EXTRACTED FROM ISCHEMIC KIDNEY
	mm. Hg	*mm.* Hg
1	4	16
2	14	23
3	15	27
4	18	38
5	25	42
6	24	24
7	−2	14
8	−1	10
9	0	12
10	0	15
11	−2	13
Averages	8.8 (±3.8)	21.3 (±3.3)

The difference of the averages is 12.5 (±4.9).

The average of the differences found in individual pairs of experiments is 12.6 (±1.7), an undoubtedly significant difference.

Extracts were prepared from both kidneys and assayed for their pressor effects upon unanesthetized rabbits by methods previously described (16).

RESULTS. Each pair of extracts consisted of the extract of ischemic kidney and that of the opposite normal control kidney. In ten of the eleven pairs the pressor effect of the extract of ischemic kidney was greater than that of the extract of normal kidney, and in the eleventh pair the pressor effect of each extract was the same. The average rise in blood pressure for the eleven extracts of the ischemic kidneys was 21 mm. of Hg, compared to 8 mm. of Hg, for the eleven extracts of the normal kidneys (table 3).

DISCUSSION. In this communication certain observations are reported upon the pressor reaction which follows the termination of temporary, complete renal ischemia, in an effort to throw light upon the nature of the hypothetical pressor substance which is presumably formed in the ischemic kidney.

The first experiment demonstrated that a previous injection of 933F reversed the pressor effect of epinephrine, but failed to abolish that following reëstablishment of the circulation in the completely ischemic kidney. It is therefore concluded that the substance responsible for the rise in blood pressure following the termination of complete renal ischemia is not epinephrine or an epinephrine-like substance.

In the second experiment it was shown that the rise in blood pressure ordinarily following termination of complete renal ischemia was abolished or markedly diminished in animals previously rendered tachyphylactic to renin. These observations prove that the substance causing the rise in blood pressure is renin or a principle having similar properties of tachyphylaxis. Until more is known concerning the nature of tachyphylaxis, however, no further conclusions can be drawn.

The finding of increased amounts of renin in completely ischemic kidneys of cats recalls previous observations showing the amount of renin to be increased in partially ischemic kidneys of hypertensive dogs. These observations would seem to indicate that as a result of total renal ischemia, renin is either formed in greater quantities than in normal control kidneys, or becomes more readily available for extraction. In this connection, reference may be made to recent studies by Goormaghtigh (19) who noted hypertrophy and hyperplasia of certain cells in the preglomerular arterioles of ischemic kidneys. It is possible that renin is formed in these cells and that their increased activity in renal ischemia may indicate augmented production of this substance.

CONCLUSIONS

1. The previous injection of piperidomethyl-3-benzodioxane (933F) does not prevent the rise in blood pressure which results from the reëstablish-

ment of the circulation in the completely ischemic kidney, proving that the substance responsible for this rise is not of an epinephrine-like nature.

2. The pressor reaction which follows the termination of complete renal ischemia is greatly reduced or abolished in animals rendered tachyphylactic to renin. This proves that the substance causing the rise in blood pressure is renin or a pressor principle having similar properties of tachyphylaxis.

3. The pressor effect of extracts of completely ischemic kidneys is greater than that of extracts of control normal kidneys.

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THE SUBSTANCE OF THE ANTERIOR PITUITARY GLAND WHICH INCREASES LIVER FAT

JAMES CAMPBELL AND H. C. KEENAN

From the Department of Physiology, University of Toronto, Canada

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It was observed (Best and Campbell, 1936, 1938) that certain extracts from the anterior pituitary gland produced a great increase in the liver fat of fasting animals. Since that time we have been interested in the purification of the active material. For this purpose a means of assaying the so-called "liver-fat" activity was developed (Campbell, 1938). The extent to which this activity is possessed by the various fractions of the anterior pituitary gland extracts and the properties of the extracts containing this active substance in highest concentration are described in this paper.

METHODS. The method of assaying the liver fat increasing activity (L.F.A.) of the anterior pituitary extracts has already been described. Ketogenic activity was determined by a method which may be outlined as follows. Male rats of 100 to 200 grams were fasted in Hopkins metabolism cages for 24 hours and then given a single injection of extract. The urinary excretion of total acetone bodies during the following 24 hours was determined by the method of Van Slyke (1917). The response produced by the unknown extract (in 5 rats) was compared with the responses produced by two doses of the standard anterior pituitary preparation (A.P.P.). After a rest period of 10 to 14 days the rats were used again. The responses apparently become more uniform after the animals have been used for testing about 3 times. Shipley and Long (1938) question the value of results obtained from the determination of urinary ketones in rats. Others, however (Gray, 1938, Neufeld and Collip, 1938), have used modifications of this method and for the purposes of assay under standard conditions we have found it to be satisfactory.

Diabetogenic activity was tested on normal young male dogs of about 10 kgm. weight. These animals were given a diet of 400 gram lean meat and 40 gram sucrose daily with an adequate supply of vitamins. Daily subcutaneous injections of extract were given. Diabetogenic activity was indicated by a rise in blood sugar to about 200 mgm. per cent in about 3 days and the appearance of glycosuria at the same time or somewhat later.

Prolactin activity was determined according to the method of Riddle,

Bates and Dykshorn (1933). Over a 4-day period the extracts were injected daily into the breast muscle of pigeons; the proliferation of the crop glands was then determined by weight and by noting the amount of crop milk which was present. The "local intradermal" crop gland response method (Lyons, 1937) has also been used. Melanophore-expanding activity was determined by injection of the extract into the dorsal lymph sacs of frogs which were exposed to light. The activity was estimated by the degree of darkening produced by the injections.

The method of preparing the A.P.P. has been described by Best and Campbell (1936) in their experiments on rats. Samples of this preparation, kept as a dry powder at 7°C., have shown no demonstrable loss of potency in 5 years. The prolactin and follicle-stimulating hormone (F.S.H.) were prepared according to the method of Bates and Riddle

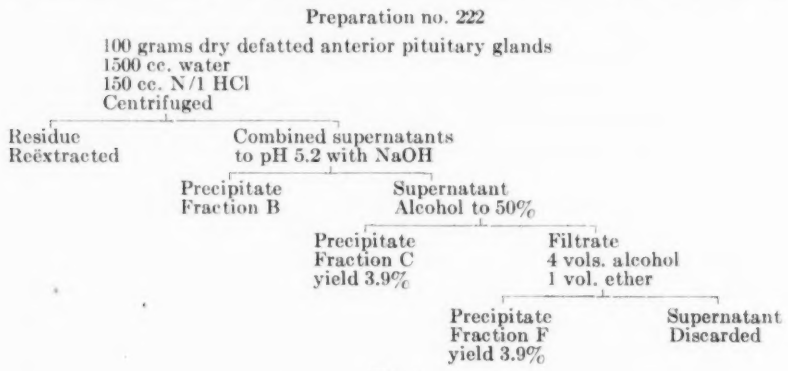

Fig. 1

(1936). The fractions referred to as mammotropic and adrenocorticotropic by Lyons (1937) were also prepared.

The extract numbered 222F was prepared from dry defatted anterior pituitary glands. The fresh glands were treated with acetone and ether to yield a dry light-colored product which was then finely ground. This dry material was extracted (fig. 1) with aqueous HCl by mechanical stirring for 3 hours. The insoluble material was separated by centrifuging and reëxtracted. The combined filtrates were brought to pH 4.5 and the precipitate removed. To the filtrate an equal volume of 95 per cent ethyl alcohol was added slowly while stirring. The precipitate was removed and to the filtrate two volumes of alcohol, each equal to the first, and 1 volume of ether were added. The precipitate (no. 222F) was collected by decanting and centrifuging, and was dried with alcohol and ether. The powder was dissolved in dilute NaOH, brought to pH 8 and the insoluble material removed by centrifuging.

For the preparation of the globulin extract the anterior lobes of the pituitary glands were dissected immediately after removal from the animal and frozen in CO_2 snow. The glands were then minced and extracted as outlined in figure 2 at temperatures not exceeding 7°C. The "diabetogenic" activity is apparently easily lost and the extract was therefore stored at 4°C. for periods not exceeding 1 week. All these extracts were prepared from the pituitary glands of cattle.

RESULTS. *Relation of liver fat activity to prolactin.* In our hands the method of Bates and Riddle (1936) has yielded the most satisfactory

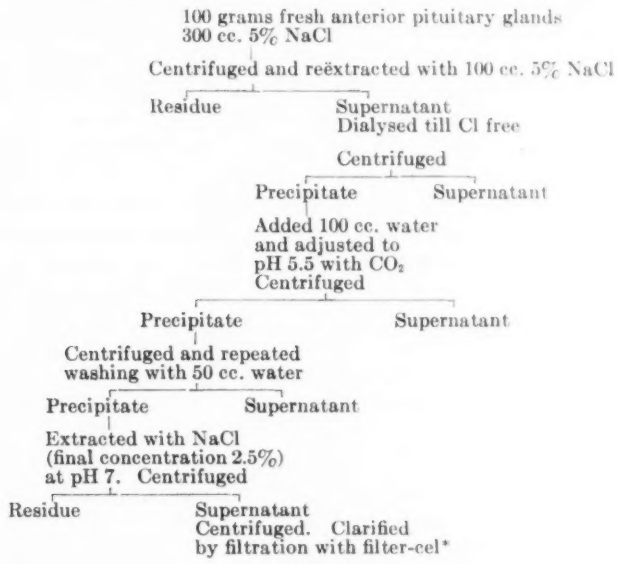

Fig. 2

*A filter aid prepared by the Johns-Manville Co.

preparations of prolactin. Four separate preparations were all highly active in producing proliferation of the crop gland in pigeons, while the A.P.P. produced only a slight crop gland response. The prolactin preparations have been tested for L.F.A. on 7 occasions and each time were found to be less active than the A.P.P. (table 1). The average of all the tests showed that the prolactin preparations had 28 per cent of the activity of the standard. Attempts were made to free the prolactin of all L.F.A. by repeated precipitation of the prolactin from dilute alkaline aqueous solution by the addition of dilute hydrochloric acid and also acetic acid to pH 4.85. A portion of the L.F.A., which remained in the filtrates, was

thus removed from the prolactin precipitate but the separation was not complete.

The F.S.H. fraction of Bates and Riddle (1936), on the other hand, showed very high L.F.A. (250 per cent) and negative prolactin activity when compared with the standard preparation.

The fractions which have been termed mammotropic and adrenocorticotropic by Lyons (1937) were prepared, but since the latter fraction had considerable crop gland stimulating potency the separation was not as complete as the author indicates. Three tests on each of these fractions showed that they contained less L.F.A. than the A.P.P. The average for the mammotropic fraction was 42 per cent and for the adrenocorticotropic fraction 77 per cent. The mammotropic fraction produced much greater crop gland proliferation than the A.P.P.

TABLE 1

PREPARATION	LIVER FAT INCREASING ACTIVITY	KETOGENIC ACTIVITY	PROLACTIN	DIABETOGENIC ACTIVITY
	per cent	*per cent*		
Standard preparation	100	100	+	Slightly active
Prolactin (acc. to Bates and Riddle, 1936)	28	9	+++++	Negative
Mammotropic (acc. to Lyons, 1937)	42		++++	
F.S.H. fraction (acc. to Bates and Riddle, 1936)	250		No activity	
Adrenocorticotropic fraction (acc. to Lyons, 1937)	77		++	
Fraction # 222F	330	17	No activity	
Globulin extract	22–100	120–500	+++++	Highly active

A sample of prolactin prepared by Doctor White of Yale University was tested for L.F.A. It was estimated (by a single test) that this material contained 30 per cent of the activity of the A.P.P. These results indicate that prolactin and L.F.A. are separable.

Relation of liver fat activity to ketogenic activity. The amounts of ketogenic and L.F.A. which were present in the globulin fraction varied considerably from one preparation to another, although the same method of preparation was used. In 4 preparations the ketogenic activity averaged 142 per cent and the L.F.A. 46 per cent (table 1). It thus appeared that in this fraction, as compared to the A.P.P., the ketogenic and L.F.A. did not occur in constant proportions. The ketogenic activity of 8 other globulin preparations was higher than in the former, averaging 319 per cent, but in these L.F.A. was not determined.

The preparation no. 222F showed the most striking difference between ketogenic and L.F.A. The L.F.A. (3 tests) averaged 330 per cent while the ketogenic activity (4 tests) averaged only 17 per cent. It appears that these two forms of activity are not necessarily associated.

Relation of liver fat activity to melanophore-expanding activity. Determination of the liver fat and melanophore-expanding activities in extracts from different parts of the pituitary gland (pars anterior, posterior, intermedia, tuberalis and pituitary colloid) showed that there was no correspondence between these activities. The melanophore-expanding activity per gram of tissue was greatest in the posterior lobe and least in the anterior, while the L.F.A. was greatest in the anterior and least in the posterior lobe extracts.

Relation of ketogenic activity to diabetogenic activity. The globulin fraction of the anterior pituitary given subcutaneously has been found to be highly active in producing transient diabetes in dogs, and in three cases permanent diabetes. This extract also produced a rapid fall in insulin content of the pancreas (Best, Campbell and Haist, 1939). In doses of 12 to 27 mgm. per kgm. per day (the upper limit being in excess of that required to produce an effect) the globulin produced glycosuria. Out of 23 dogs, 20 showed distinct glycosuria and 3 gave no response. This fraction is therefore high in diabetogenic activity. Although this test was not quantitative, it appeared that diabetogenic activity was associated with ketogenic activity as repeated tests on the globulin fraction indicated that a parallelism existed between them.

Relation of prolactin to diabetogenic activity. The "globulin" fraction was the only one of all those tested which produced hyperglycemia and glycosuria with any regularity in normal dogs. This extract was also highly active in producing crop gland proliferation so that in this case diabetogenic activity has not been separated from prolactin activity.

The diabetogenic activity of prolactin prepared by the method of Bates and Riddle has been tested in 3 normal dogs given large doses of extract. The prolactin produced good proliferation of the crop gland and was estimated to contain 1 to 2 units (Riddle) per mgm. Dog 1, a male of 9.1 kgm., was injected daily for 17 days with an average of 77 mgm. prolactin per kgm. No significant rise in urinary sugar occurred except on the 7th day when 3.85 grams were excreted. Dog 2, a male of 9.6 kgm., received 13 injections averaging 40 mgm. per kgm. per day but no rise in urinary sugar occurred. Dog 3, a female of 7.5 kgm., received 16 injections of 97 mgm. per kgm. per day. The mammary glands hypertrophied and lactation ensued about the 10th day but no change in sugar excretion was observed.

These experiments indicate that prolactin is not necessarily associated with diabetogenic activity and therefore support the findings of Young (1938), Shipley and Long (1938) and Houssay and Biasotti (1938).

Relation of prolactin to ketogenic activity. The ketogenic activity of the prolactin prepared according to Bates and Riddle was low (table 1) when compared with the A.P.P. Since the prolactin activity of the A.P.P. was low it appears that ketogenic activity is separable from prolactin activity.

Nature of the material which increases liver fat. The substance which increases liver fat is soluble in water in the presence of M/20 NaCl at pH 7. The region of minimum solubility occurs about pH 4.8 (reaction adjusted with HCl) at which point most of the active substance is precipitated. It is soluble in 65 per cent ethyl alcohol at pH 9 and at pH 3, but is insoluble at pH 5 to 7.

TABLE 2

Liver fat determined 7 hours following the administration of extract to fasting female mice

Series A. A.P.P. incubated for 4 hours at 37°C., pH 2.5 with pepsin.

Series B. A.P.P. incubated for 4 hours at 37°C., pH 2.5 with boiled pepsin.

Series C. Saline.

Series D. A.P.P. incubated in phosphate buffer for 3½ hours at 37°C., pH 8.2 with trypsin.

Series E. A.P.P. incubated in phosphate buffer for 3½ hours at 37°C., pH 8.2 with boiled trypsin.

Series F. Saline.

SERIES	DOSAGE OF A.P.P. PER MOUSE	NUMBER OF MICE	AVERAGE WEIGHT	LIVER FAT		
				Per cent	Mgm. per 100 grams body weight	Increase over control
	mgm.		*grams*			
A	7	20	18	5.69	377.	9.
B	7	20	18	9.00	709	441
C	0	10	19	5.02	368	0
D	10	20	17	4.62	278	24
E	10	20	17	12.4	762	510
F	0	10	17	4.47	252	0

We have been unable to produce definite gains in the activity of fractions which increase liver fat by fractionation with ammonium sulphate. This has been investigated by step-wise increases in the concentration of ammonium sulphate, or solution of material precipitated by higher concentrations with lower concentrations of ammonium sulphate. The active material is, however, completely precipitated by 60 per cent of saturated ammonium sulphate from pH 7 to 5.

Experiments (table 2) have shown that the L.F.A. is rapidly destroyed by digestion with pepsin and trypsin at 37°C. for 4 and 3½ hours respectively. The active material is therefore protein-like or is combined with protein material.

The curves for the inactivation of L.F.A. at 58°C. (fig. 3) show that the point of maximum stability is in the neighborhood of pH 3.5 and also that the substance is fairly heat-stable. Tests have shown that the ketogenic substance is most stable about pH 8.0 and is rapidly destroyed at pH 3.5

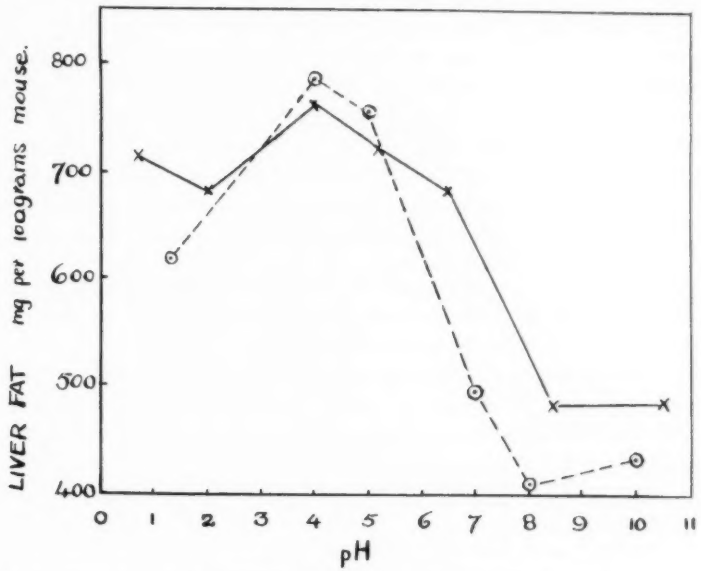

Fig. 3. Curve x——x Solutions heated at 58°C. for 21 hours. With unheated solution liver fat = 815. Curve ⊙- - -⊙ Solutions heated at 58°C. for 22 hours. With unheated solution liver fat = 968.

TABLE 3

Ketone body excretion—mgm. per rat, 5 rats per group

Temperature at which the solutions were heated = 58°C.

	TIME OF HEATING		
	30 minutes	42 minutes	110 minutes
pH 3.5	2.1	1.6	No response
pH 6.5	4.1	16.7	No response
pH 8.0	8.9	37.8	2.0

(table 3). In addition, the ketogenic substance appears to be more heat labile than the liver fat increasing substance since the former is almost completely inactivated in 2 hours at 58°C. and pH 8.0. The latter, however, still retains a large part of its activity after 22 hours at 58°C. and pH 3.5.

Discussion. It is apparent that the prolactin activity of anterior pituitary extracts does not parallel liver fat activity (L.F.A.). However, none of the prolactin preparations tested were entirely free from L.F.A. Although this is probably the result of incomplete purification it is necessary to consider the possibility that pure prolactin may be able to increase liver fat, although in much less degree than other preparations. It has also been kept in mind that the procedures of fractionation may alter the properties and the activities of the native proteins present in the anterior pituitary gland. Bates and Riddle (1936) found that prolactin is most stable to heat at pH 8.0, while we have shown that L.F.A. is most stable at pH 3.5. This is another indication that these substances are distinct.

It was previously stated that samples of prolactin and F.S.H. received from Doctor Riddle (Campbell, 1936) did not increase the liver fat of rats in doses of 20 mgm. and 5 mgm. per rat respectively. Later work demonstrated (Best and Campbell, 1938) that rats were relatively resistant to this effect and that if larger doses had been given the F.S.H. fraction would probably have increased liver fat in this species.

We were surprised to find that the liver fat activity did not parallel ketogenic activity, since there is much physiological evidence to support the view that, under certain conditions, the processes governing ketogenesis and increase in liver fat are linked together (Geelmuyden, 1923; Leathes and Raper, 1925). The results of the fractionations, however, indicate that these two effects are not due to the same substance. This is supported by the finding that L.F.A. is most stable to heat at pH 3.5 while ketogenic activity is most stable about pH 8.0. In addition, the ketogenic activity is more rapidly destroyed by heat than the L.F.A.

The high ketogenic activity of the globulin fraction appeared to be paralleled by high diabetogenic activity, so that these activities may be associated. Both are readily inactivated by heat. In this connection Shipley and Long (1938) observed that growth, glycosuric and ketogenic activities accompanied one another during the fractionation of anterior pituitary extracts. At the present time we do not know of any extract possessing higher diabetogenic activity than the "globulin" fraction.

In other laboratories (Young, 1938; Shipley and Long, 1938; Houssay and Biasotti, 1938) it has been found that prolactin is not diabetogenic. This is supported by our results, since large doses of prolactin administered to normal dogs did not produce signs of diabetes. The prolactin preparations were low in ketogenic and high in crop gland stimulating activity. This is in agreement with the previous observations of Shipley and Long (1938) who concluded that prolactin was not "of chief importance" in the production of ketogenesis by anterior pituitary extracts.

SUMMARY

1. From fractionation of anterior pituitary extracts it has been found that liver fat activity is not necessarily associated with prolactin, ketogenic or melanophore-expanding activity. Prolactin is not associated with ketogenic or diabetogenic activity, but the latter two appear to run parallel during fractionation.

2. Liver fat activity is rapidly destroyed by pepsin and trypsin.

3. Liver fat activity is most stable to heat at pH 3.5.

4. Ketogenic activity is most stable to heat at pH 8.0 and is more readily destroyed than liver fat activity.

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THE INFLUENCE OF ADRENALECTOMY AND OF FASTING ON THE INTESTINAL ABSORPTION OF CARBOHYDRATES

ROSE MARRAZZI

From the Department of Biology, Washington Square College, New York University and the Department of Pharmacology, New York University College of Medicine

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An integral part of the picture of adrenal insufficiency, as Verzar describes it (1936, 1939), is the failure of selective intestinal absorption of metabolizable sugars. This assertion rests upon studies made by Wilbrandt and Lengyel (1933) in Verzar's laboratory on the absorption of glucose and of a nonmetabolizable sugar, xylose, in adrenalectomized rats. They found, in 13 adrenalectomized rats, that 41 per cent of the glucose placed in intestinal loops was absorbed in 1 hour as compared to 71.6 per cent absorbed by normal animals, whereas, in the case of xylose, they found no difference between the normal and the adrenalectomized rats. Further work from Verzar's laboratory claims the same to be true for cats (Issekutz, Laszt and Verzar, 1938) and for frogs (Minibeck, 1939). Cori had also previously (1927) reported a decrease in absorption of glucose in adrenalectomized rats. Deuel, Hallman, Murray and Samuels (1937) do not find a decrease. Althausen, Anderson and Stockholm (1939) agree with Deuel et al. only if a salt supplement is added to the diet; but if, on the other hand, the salt supplement is withheld, they confirm Verzar.

We were impressed, as have been others (Verzar, 1936; Marrian, 1937), by the significance it would have if it were to be established that an underlying difficulty in adrenal insufficiency is the failure of phosphorylation of glucose by the mucosa of the intestine resulting in a decrease in absorption rate to that of purely nonselective physical processes. The osmotically active glucose might, under these circumstances, cause a shift of water and electrolytes from the body fluids and tissues into the intestinal lumen, such as occurs into the peritoneal cavity following an intraperitoneal injection of glucose (Schechter et al., 1933; Gilman, 1934; Darrow and Yannet, 1935). The diarrhea which occurs in many adrenalectomized animals could also be explained in this way.

We therefore undertook to study again the intestinal absorption of sugars in adrenalectomized rats. I wish to express my thanks to Prof. Robert Gaunt for his interest and help in this work.

METHODS. In testing Verzar's contention it seemed desirable not to alter the technique adopted in his laboratory which utilizes intestinal loops, despite the objections inherent in the method. These are principally the use of anesthesia and the trauma involved in laparotomy and manipulation of the intestine. Cori's technique (1925) of administering the glucose by stomach tube to unanesthetized animals avoids these objections but introduces new difficulties, such as the influence of variations in gastric and intestinal motility and emptying time on the absorption from the gut.

Absorption was studied in adrenalectomized, in sham operated, in non-operated fasted and in non-operated non-fasted rats. Male rats weighing 200 to 300 grams were used. Stock diet was allowed ad lib and a record of the daily intake was kept. Adrenalectomies were performed three days before the experiment and the rats immediately given access to 0.9 per cent saline as drinking fluid. This was continued until absorption was determined. Some of the controls, likewise, were given saline for three days before determining absorption. Others received tap water. When adrenalectomized animals received only tap water to drink during this three day period, they were unable to withstand the stress of the subsequent experimental procedure. The adrenalectomized and control animals were fasted for the 48 hours before absorption was determined.

The animals were anesthetized with intraperitoneal injections of 0.05 cc. of nembutal (6.5 per cent solution) per 100 grams' body weight. The adrenalectomized animals were more sensitive to the nembutal and required only $\frac{2}{3}$ of this dose. The sham-operated rats required the full dose. The abdomen was opened by midline incision and a loop of gut was measured off starting at the beginning of the jejunum. Measurements were accurately checked after the death of the animal. The absorption was calculated as milligrams per inch of gut.

The loop was cannulated at both ends and gently washed with warm 0.9 per cent saline. The abdomen was closed with clamps and the animal allowed to rest for one hour. The animal was kept warm during the entire experiment by means of hot water bottles. At the end of the hour, 3 cc. of isotonic sugar solution were introduced into the loop and the rubber tubing at the ends of the cannulae clamped so that there could be no leakage.

After exactly $\frac{1}{2}$ hour the unabsorbed solution was removed and the gut washed three times with 3 cc. portions of normal saline. These washings were added to the recovered solution and the whole analyzed in duplicate for sugar by the Hagedorn-Jensen method.

In order to aid in analysis of our results the following further controls were used. Rats were subjected to sham operation. This consisted of exposing the adrenals by bilateral lumbar incisions. In some cases the adrenals were also gently freed from the kidney and the surrounding fat was removed. In others the adrenals were exposed but not disturbed.

Twenty-four hours later the food was removed, and the animals fasted for 48 hours before absorption of glucose was determined. Some of this group were given saline and some, tap water as drinking fluid. Other rats which underwent the same operative procedure were allowed to survive and were followed for over two months during which time they showed no signs of adrenal insufficiency.

We also compared the absorption of glucose and of xylose in normal animals, which had been allowed the usual stock diet up until 2 to 4 hours before the experiment, with that in normal rats which had been fasted for 48 hours. In addition, 4 rats were allowed 7 grams of food each (i.e., the

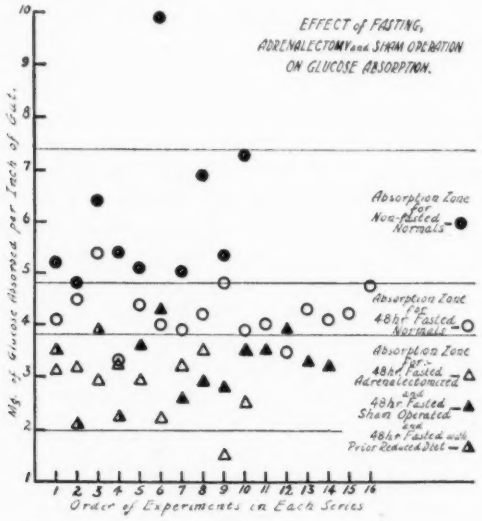

Fig. 1

average amount taken by the adrenalectomized or sham-operated rats during the 24 hour period following operation) for a period of 24 hours. Following this the food was removed entirely and the rats fasted for 48 hours. This made their food intake equal and comparable to that of the sham-operated and adrenalectomized rats for the 72 hour period preceding the experiment. Then absorption was determined as in the other rats.

Results. The results divide themselves into two classes. One consists of the values for the absorption of glucose, which vary with the conditions set; and the other, of the values for absorption of the non-metabolizable sugar, xylose, which remain fixed under the various experimental conditions.

The scatter diagram in figure 1 includes all the results on the absorption

of glucose. They are seen to fall into three distinct zones with only a small degree of overlap. The zone of maximum absorption is occupied by the values for the non-fasted normal animals. An intermediate zone is constituted by the values for 48-hour fasted normal animals, while in the zone of minimum absorption are fairly evenly interspersed the values for both adrenalectomized and sham operated 48-hour fasted animals. Also in this group are found the values on four animals which, previous to a 48 hour fast, had had their food intake restricted to the level to which that of adrenalectomized and sham-operated animals drops voluntarily following operation.

The lack of any corresponding effect of adrenalectomy on xylose absorption is illustrated in figure 2. The lack of influence of fasting on xylose absorption is shown in figure 3. These rats are a later series than those in figure 2, and show a higher absorption rate, but there is no difference between the non-fasted and fasted rats studied at the same time within the

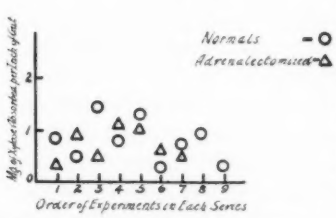

Fig. 2. Non-effect of adrenalectomy on xylose absorption

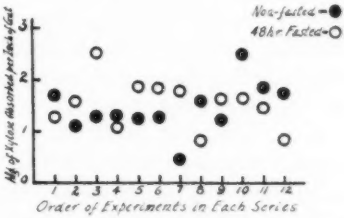

Fig. 3. Non-effect of fasting on xylose absorption

series. Variations in different series have also been observed for fructose absorption (Cori and Cori, 1928).

In no group is there any difference between those rats receiving tap water and those receiving saline as drinking fluid in regard to absorption of either glucose or xylose. (This does not include the adrenalectomized rats since they could not be maintained on tap water.)

Among the sham-operated rats there was no difference in absorption between those animals in which the adrenals had been merely exposed, and those in which these glands had been freed from the kidney and perirenal fat.

The normal average daily food intake was 13.5 grams. This dropped to an average of 7 grams the day following adrenalectomy or sham operation.

Thus adrenalectomy, sham operation or simple fasting reduces the absorption of glucose from the jejunum but does not affect that of xylose.

Discussion. The results of these experiments agree with the findings from Verzar's laboratory (Wilbrandt and Lengyel, 1933) in that the absorp-

tion of glucose is diminished after adrenalectomy, though to a smaller extent than they report, while the absorption of xylose is not affected. Our experiments show too, however, that fasting also limits subsequent absorption of the preferentially absorbed sugar, glucose, but not of xylose. In fact, it seems that fasting could account for the effect of adrenalectomy on the absorption of carbohydrates. We have shown that on the day following operation, adrenalectomized and sham-operated animals eat on the average about 7 grams of food instead of their usual average of 13.5 grams. This suggests that it is the post-operative anorexia that accounts for the decrease in absorption to below that of 48 hour fasted non-operated rats, by prefixing a period of subnormal intake, and thus in effect increasing the total period of fast. This is supported by the same low level of absorption obtained in animals in which the same period of subnormal intake was enforced preceding the 48-hour fast, but which were not subjected to either adrenalectomy or sham operation.

The findings on sham-operated animals appear to be at variance with those of Wilbrandt and Lengyel but, since the length of the post-operative interval and the regime on which the rats were maintained are not given, it is difficult to interpret their experiments.

The results of Deuel et al. (1937), who studied absorption by the Cori technique, in rats 12 to 20 days post-operatively, may not be strictly comparable to those reported here because of the long post-operative period intervening in their experiments, whereas ours were obtained three days post-operatively to conform to the experiments of Wilbrandt and Lengyel. Nevertheless in Deuel's experiments the effect of fasting on absorption does not seem to have been sufficiently taken into account. Judging from our results, as well as from those of Cori (1927), the extra 32-hours fast to which the controls were subjected might have lowered the absorption rate to that of the adrenalectomized rats which were fasted only 16 hours. Interpreted in this way their findings are not in disagreement with those reported here.

The present results disagree with the view of Althausen et al. (1939) that sodium chloride administration removes the difference in glucose absorption between adrenalectomized and control animals, since in our experiments the difference was obtained despite the sodium chloride supplement. Althausen's adrenalectomized animals had access to sucrose for the first 8 hours of the "fast" period, the amount taken being determined presumably by their appetite. The fasting period in these adrenalectomized animals is therefore obviously less than that in the controls, and this would modify the results as pointed out in the discussion of Deuel's work. Accordingly, had the sodium chloride restored the absorption to normal, then those animals the fast of which was shortened by sucrose, though adrenalectomized, should have shown a higher absorption rate than the controls. The

effectiveness of the carbohydrate fraction of the previous diet in influencing carbohydrate absorption has been shown by Westenbrink (1934, 1936). In explaining the apparent effect of sodium chloride, it seems likely that this was indirect—i.e., the well known one of diminishing anorexia. Consequently, the rats on sodium-chloride supplement probably ate more food through the entire post-adrenalectomy period and were able, as well, to take better advantage of the sucrose allowed for the first 8 hours of the "fast". No figures are given for the amounts of the food and sucrose intake so that the extent of these effects cannot be accurately gauged.

The difference between the results of the last two groups of investigators and those reported here cannot be attributed to differences between the Cori technique and ours, since Cori has also reported a diminution in absorption in adrenalectomized rats (1927).

The definite decrease in absorption of glucose, though not of xylose, in adrenalectomized animals, even when supported by saline, closely resembles the decrease in control animals the food intake of which has fallen or been reduced to that of the adrenalectomized animals. This suggests that the decreased absorption may be attributed as convincingly to the effects of anorexia as to a primary disturbance in the mechanism of absorption.

SUMMARY AND CONCLUSIONS

1. The absorption of glucose, a metabolizable sugar, and of xylose, a non-metabolizable sugar was studied in adrenalectomized, in sham-operated, in non-operated fasted, and in non-operated non-fasted rats.

2. The absorption of glucose in adrenalectomized rats even though on a sodium chloride supplement was decreased, whereas that of xylose was not.

3. Sham-operated rats showed a like decrease in the absorption of glucose.

4. The food intake of both adrenalectomized and sham-operated rats was found to decrease post-operatively.

5. Restriction of food intake or fasting was found in itself to decrease absorption of glucose but not of xylose in non-operated animals.

6. It appears that the reduction of food intake, induced by post-operative anorexia, and the surgical manipulation can account for the decrease in the absorption of glucose in adrenalectomized animals. It is probable, in fact, that anorexia alone can account for it.

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CONTROL OF CORONARY BLOOD FLOW IN THE HEART-LUNG PREPARATION

ERICH HAUSNER,[1] HIRAM E. ESSEX, J. F. HERRICK AND EDWARD J. BALDES

From the Division of Experimental Medicine and Division of Physics and Biophysical Research, The Mayo Foundation, Rochester, Minnesota

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Recently Wiggers (1) has discussed the currently accepted views on the principal factors affecting coronary circulation. Changes in aortic pressure, the vasomotor mechanism, the action of metabolites and mechanical influences such as the massaging action of the myocardium on the cardiac vessels, are considered by different investigators as the chief mechanisms responsible for the regulation of coronary blood flow.

The desirability of further work on the control of coronary blood flow in the heart-lung preparation was indicated by a few preliminary experiments done in this laboratory. Since some of the results obtained were not in complete accord with the literature it seemed advisable to reinvestigate certain phases of the problem with a method of measuring blood flow not previously used in experiments of this nature.

We are reporting here the results of a study on the influence of 1, changes in the mean arterial blood pressure; 2, changes in cardiac output; 3, stimulation of the cardiac branches of the stellate ganglion, and 4, changes in the heart rate on the coronary blood flow of the heart-lung preparation of the dog.

METHODS. Previous investigators working with the heart-lung preparation measured coronary sinus outflow by means of a Morawitz-Zahn cannula or determined coronary inflow by the use of the hot wire anemometer, which was connected to a cannulated perfused coronary artery. In the present experiments coronary inflow was measured with the thermostromuhr which caused a minimal disturbance of the heart and its vessels (2, 3). The heart-lung preparation was made under pentobarbital sodium (nembutal) and the thermostromuhr unit was applied to the circumflex branch of the left coronary artery either before or after the heart-lung preparation was completed. Application of the unit before preparation of the heart and lungs facilitated obtaining hemostasis. Twenty-three heart-lung preparations were made. The operative pro-

[1] Now residing in Amsterdam, New York.

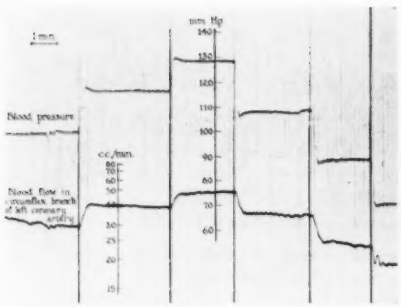


Fig. 1

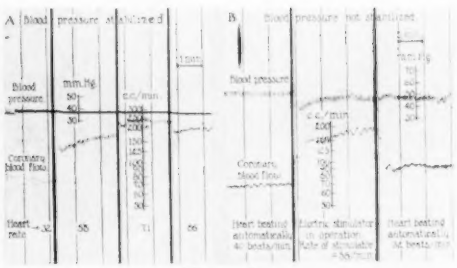


Fig. 4

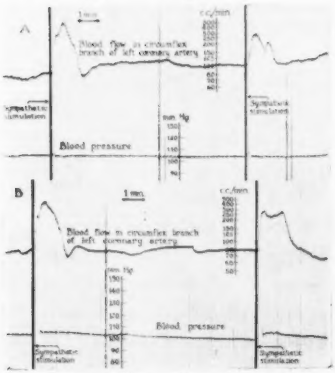
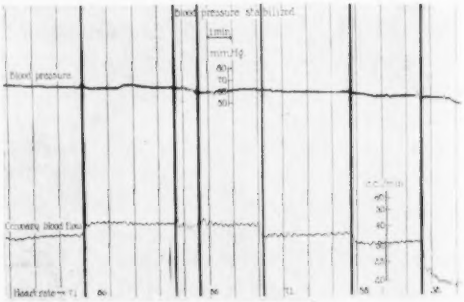


Fig. 2



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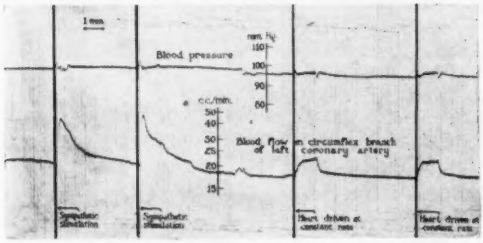

Fig. 3

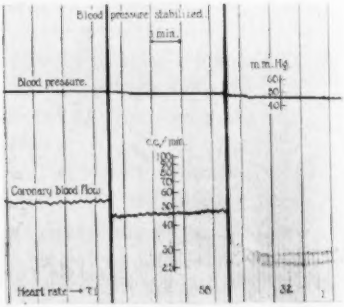

Fig. 6

Figs. 1-6

Fig. 1. Photographic record showing the effect of changes of blood pressure on the coronary blood flow of the heart-lung preparation. The blood flow in all the experiments was measured in the circumflex branch of the left coronary artery.

Fig. 2. Upper tracing, photographic record of coronary blood flow. Lower tracing, record of blood pressure. At the point indicated by the heavy vertical lines the cardiac branches of the stellate ganglion were stimulated (inductorium). Blood pressure in A was kept constant but about fifteen seconds were required for equilibrium of blood pressure in B.

cedures which usually lasted one and a half hours included setting up the heart-lung, applying the thermostromuhr unit to the coronary artery and the preparation of the sympathetic nerves for stimulation on one or both sides. The sympathetic nerves were prepared for stimulation by sectioning all of the branches of the stellate ganglion except the cardiac branches (ansa Vieusseni). Cardiac output was measured directly; the blood pressure and coronary flow were optically recorded. The arterial blood pressure was controlled by a method similar to that described by Greene (4).

Blood pressure and coronary flow. In analyses of the dynamics of coronary circulation in the denervated heart-lung preparation, arterial blood pressure has repeatedly been demonstrated to be the chief factor influencing coronary blood flow (5–10). In repeated experiments we have likewise found that an increase in the mean arterial pressure of the heart-lung preparation caused an augmented coronary flow and a decrease of blood pressure resulted in a diminished coronary flow (fig. 1). By use of another method of measuring coronary flow mean arterial pressure has again been shown to be an important and determining factor in the control of coronary flow in the denervated heart-lung preparation as the data of a typical experiment indicate (table 1).

Cardiac output and coronary flow. By keeping the blood pressure and temperature of the perfusing blood constant, the uncomplicated effect of changes in cardiac output on coronary flow was studied. The venous inflow into the heart was increased or decreased at will by raising or lowering the blood reservoir. In a compensating heart an increased output resulted from the former.

Our results confirm the observations of previous workers (5, 11, 12). Augmenting cardiac output as much as 275 per cent above the basal level did not increase coronary flow more than 3 to 6 per cent. This increase, however, is well within the limits of the error of the method and cannot

Fig. 3. Upper tracing, photographic record of blood pressure. Lower tracing, photographic record of coronary blood flow. The first and second heavy vertical lines indicate the points at which the cardiac branches of the stellate ganglion were stimulated. At the third and fourth heavy vertical lines the heart was accelerated from 164 to 212 beats per minute. The blood pressure was controlled.

Fig. 4A. The effect on coronary blood flow of driving the heart at successive increased rates, the blood pressure being kept constant; B, the effect of imposing an increased rate on the automatically beating heart with the blood pressure uncontrolled.

Fig. 5. An increment in the imposed rate of the heart above 86 beats per minute did not increase the coronary blood flow but each successive decrement in heart rate reduced the coronary blood flow.

Fig. 6. Decrements in the imposed rate of the heart resulted in significant decrements in coronary flow.

be explained in our experiments by changes in mean arterial blood pressure since the latter was constant. Even sudden marked changes of cardiac output in the denervated heart did not influence the coronary flow, in contrast to the effect which increase of cardiac output is reported to have on coronary flow in the innervated heart. According to Anrep, the increase in coronary flow with increased cardiac output in the innervated heart is of reflex origin and disappears after section of the vagi.

Influence of vasomotor nerves on coronary flow. It is evident from the work of many that the coronary circulation in the intact animal is affected by factors in addition to the influence of the mean arterial blood pressure. According to numerous researches nervous influences play a prominent rôle in this regard. Wiggers recently reviewed the subject critically and discussed the difference in opinion of various investigators concerning the

TABLE 1

Relation of changes in coronary flow and cardiac output to changes in blood pressure

	BLOOD PRESSURE		CORONARY FLOW		CARDIAC OUTPUT	
	Mm. Hg	Per cent increase	Cc. per minute	Per cent increase	Cc. per minute	Per cent increase
Control values	70		25		132	
Values after blood pressure was increased 30 mm. Hg	100	+43	33	+32	220	+67
Values after blood pressure was increased 45 mm. Hg	115	+64	41	+64	194	+47

effect of sympathetic and parasympathetic stimulation on the coronary vessels.

Sympathetic stimulation increases markedly the force of left ventricular contraction and raises the arterial pressure. Increased arterial pressure alone augments coronary flow and the augmentation of coronary flow on sympathetic stimulation could be attributed to a summation of both factors. Wiggers has stressed the importance of changes in blood pressure and heart rate resulting from vasomotor stimulation. The early workers assumed that the peripheral resistance of the heart-lung apparatus represented the blood pressure of the preparation but when the dynamics of the heart are rapidly changing as with sympathetic stimulation, this assumption is erroneous. In order to study the effect of the vasomotor action of the sympathetic nerves, the mean arterial pressure must be controlled. To accomplish this in our experiments a rubber tubing was joined to the arterial side of the heart-lung preparation and connected with a flask, which could be raised to a height of about 2 meters. The surplus of blood ejected during the increase in force of the ventricular contraction

was carried into this flask. The mean arterial blood pressure was effectively equalized by this method (4). The blood pressure usually remained on a stabilized level during sympathetic stimulation (fig. 2A). Occasionally at the beginning of stimulation a few seconds were required for the blood pressure to come to equilibrium (fig. 2B).

In the present series of experiments the cardiac branches of the stellate ganglion of one side or the other were stimulated by means of an inductorium. The strength of the current was varied by changing the position of the induction coil from 12 to 4 cm. Sympathetic stimulation was usually carried out in the beginning because sometimes the heart failed to respond to the stimulus later in the experiment. The sympathetic nerves on one side were usually stimulated for thirty seconds. Coronary flow

TABLE 2

Effect of sympathetic stimulation on coronary flow and cardiac output under uncontrolled blood pressure

	HEART RATE		UNCONTROLLED BLOOD PRESSURE		CORONARY FLOW		CARDIAC OUTPUT	
	Beats per minute	Per cent increase	Mm. Hg	Per cent increase	Cc. per minute	Per cent increase	Cc. per minute	Per cent increase
Control values	138		76		57		92	
Values resulting from sympathetic stimulation for thirty seconds	150	+8.7	82	+7.9	300	+426	268	+191
Control values	150		75		25.5		226	
Values resulting from sympathetic stimulation for thirty seconds	174	+16	104	+39	52.5	+106	592	+162

before and after stimulation was continuously photographed and the heart rate and cardiac output were determined. In every instance stimulation of the sympathetic nerves was followed by a large augmentation of coronary flow. In none of our experiments was a decrease in coronary flow observed, regardless of the strength of stimulus. Increases in coronary flow on stimulation of the cardiac branches of the stellate ganglion varied from values of 28 to 426.0 per cent. On the average, the increase was about 150 per cent. The percentage increase in flow on stimulation of the sympathetic nerves reached similar high values when mean arterial blood pressure was kept constant. Data on changes in coronary flow with and without blood pressure control are presented in tables 2 and 3.

Morawitz and Zahn (8), in their experiments, noted that the coronary outflow on sympathetic stimulation showed a much greater increase than could be explained by the accompanying increase in blood pressure.

We are well aware of Wiggers' findings that mean arterial blood pressure can remain constant and that significant changes in pulse pressure may alter coronary flow. In our experiments, the cannulated innominate artery carried the blood from the heart and the rubber tubing of the equalizer was connected distal to this branch of the aorta. Mean blood pressure was recorded optically from a needle inserted in the tubing near the arch of the aorta. The increase in magnitude of ventricular contraction and the changes in systolic and diastolic arterial pressure, mean pressure remaining unaltered, possibly may have been an additional factor in the augmentation of coronary flow, but we question whether increases of more than 400 per cent were owing to possible changes in pulse pressure alone. As shown in the next section of this paper, increases in heart rate produced by electrical means are capable of causing a significant

TABLE 3

Effect of sympathetic stimulation on coronary flow and cardiac output under controlled blood pressure

	CONTROLLED BLOOD PRESSURE	CORONARY FLOW		CARDIAC OUTPUT	
		Cc. per minute	Per cent increase	Cc. per minute	Per cent increase
	mm. Hg				
Control values	104	100		314	
Values resulting from sympathetic stimulation for thirty seconds	104	350	+250	380	+21.0
Control values	103	123		268	
Values resulting from sympathetic stimulation for thirty seconds	103	350	+184.6	282	+5.2

augmentation in coronary blood flow. However, when all the known factors are considered it is difficult to account adequately for the results of sympathetic stimulation in the heart-lung preparation without invoking vasodilatation of the coronary vessels.

Influence of heart rate on coronary flow. By changing the temperature of the perfusing blood, by cooling or warming the sinal node, or by stimulating the sino-auricular node by an electrical stimulator the heart rate can easily be changed.

Porter (7) and Langendorff (6) explained increases in coronary flow due to increases in rate on the basis of the massaging action of the heart. Morawitz and Zahn (8) accelerated the heart beat by warming the sinal node and observed a 50 per cent decrease in flow from the coronary sinus. Cooling the sinal node decreased arterial pressure and coronary flow. The decrease in coronary flow during acceleration of the heart rate could

not be entirely explained, in the opinion of these writers, by the fall in blood pressure. Nakagawa (13) noted, in his experiments, that a decrease in temperature from 38°C. to 31°C. was accompanied by a decrease in heart rate from 192 to 120 beats per minute and, simultaneously, coronary sinus output was increased from 27 cc. to 43 cc. per minute. In a second series of Nakagawa's experiments, the sino-auricular node was stimulated with single induction shocks. The venous inflow, the blood pressure, and the temperature of the perfusing medium were kept constant. In these experiments, in which heart rate was changed from 102 beats to 132 beats or from 156 to 186 beats per minute, coronary flow remained constant during stimulation. Hammouda and Kinosita (14) confirmed, in experiments on the isolated heart, the observations in the heart-lung preparation—that the coronary flow, even within wide ranges of heart rates, is not altered, and only excessive acceleration leads to decreased flow.

Miller, Smith and Graber (15) studied the influence of acceleration of the heart beat on coronary flow in the isolated heart and the heart in situ. The isolated heart of a rabbit was stimulated with rhythmically induced shocks at different rates. Blood pressure and temperature were kept constant. The coronary sinus flow was measured with a Morawitz-Zahn cannula. Acceleration of cardiac rate from 120 to 176 beats per minute increased coronary flow about 28 per cent. Acceleration in rate from 176 to 196 beats per minute augmented coronary flow about 4.5 per cent. While acceleration of the heart beat always was followed by increased coronary flow, a greater increase was obtained if the initial cardiac rate was approximately 120 beats per minute and the heart accelerated at least 50 beats per minute. The increase in coronary flow during acceleration was less pronounced if the heart rate, before the stimulation, was about 150 beats per minute.

In a second series of experiments on the intact heart the authors just named applied small thin rubber bags to the sino-auricular node. Changes from cold to warm increased heart rate and coronary flow.

Anrep and Häusler (16) studied the effect of increased heart rate on coronary flow with the hot wire method. The rate of ventricular contraction was at first reduced by cooling the sino-auricular node or cooling the whole heart. The ventricle then was stimulated with single induction shocks and the heart accelerated from the initial rate to rates at which it did not follow all stimuli with regular contractions. The effect of acceleration is considered by these authors to depend on the change of duration and strength of premature ventricular contractions, and the coronary flow is determined by the sum of the two factors. The result may lead to an augmentation or diminution of coronary flow. In later experiments, Anrep (17) did not find any significant change during acceleration of the heart in the heart-lung preparation.

Hochrein, Keller and Mancke (18), studying coronary flow with Anrep's hot wire method and Broemser's tachograph, found that warming the sino-auricular node often increased coronary flow to values as high as 100 per cent even if the heart rate was not accelerated.

In the first series of our experiments, the heart rate was changed from the automatic rate to a different frequency by means of the electric stimulator of Hill (19). One electrode was placed near the sino-auricular node in the right auricle; the second electrode was applied near the root of the aorta. The heart was stimulated for thirty seconds and only occasionally for one minute. Heart rate and cardiac output were determined and coronary flow and the uncontrolled blood pressure were photographed throughout the experiment.

TABLE 4

Effect of acceleration of heart rate on coronary flow and cardiac output

	UNCONTROLLED BLOOD PRESSURE		HEART RATE		CORONARY FLOW		CARDIAC OUTPUT	
	Mm. Hg	Per cent increase	Beats per minute	Per cent increase	Cc. per minute	Per cent increase	Cc. per minute	Per cent decrease
Control values	100		124		58		344	
During stimulation with interrupter	100	0	148	+19.4	76	+31	248	−28
Control values	101		132		60		328	
Stimulation with interrupter	101	0	164	+24.2	82	+36.7	256	−22
Control values	93		176		52		208	
Stimulation with interrupter	97	+4.3	200	+13.6	77	+48.1	152	−27

In thirty-six observations on the heart-lung preparation, in which an accelerated rate was imposed on the heart, the coronary flow showed a marked and definite augmentation. Typical examples are given in table 4.

Usually the coronary flow increased from 30 to 50 per cent during the time of stimulation. Occasionally the increase in coronary flow was as high as 150 per cent, but never reached the average values which were obtained on stimulation of the sympathetic nerves. The coronary flow increased only during the time of the stimulation and returned to near the previous level as soon as the heart rate returned to the control value (figs. 3 and 4B).

In a few of our experiments a decrease instead of an augmentation of coronary flow was observed during the period of acceleration. In almost all of such experiments, a drop in blood pressure was noted. However, in some of these experiments, in spite of a decrease in blood pressure, the coronary flow was augmented. Many times considerable difficulty

was experienced in getting the heart to follow the rate of stimulation. Such experiments were considered failures and were not included in this report.

The response in coronary flow to acceleration of heart rate could be elicited throughout the course of the experiments. Whether the heart was driven early or late in an experiment was of importance only because coronary flow progressively increased with the duration of the heart-lung preparation. This has been a common observation of all of those who have worked with this preparation.

The experiments just described show that the coronary flow is increased when the rate of the automatically beating heart is accelerated by means of Hill's stimulator. It seemed important to determine whether changes in the induced rate of the heart would cause corresponding changes in the coronary blood flow. Therefore, another series of observations was made in which the heart rate was controlled throughout the experiment. The effect on coronary flow of increasing and decreasing the heart rate was observed while all other factors were kept constant. As a result of these observations it can be stated that within limits which vary with different preparations, increases in heart rate cause significant increments and decreases in heart rate result in definite decrements in coronary blood flow as will be indicated by the following data.

In the experiment now being described the heart was caused to beat 32 times per minute and the coronary flow was 70 cc. per minute. The rate of the heart was increased to 58 beats per minute which caused an increase in coronary flow to more than 150 cc. per minute. An increase in rate to 71 beats per minute resulted in the coronary flow reaching nearly 250 cc. per minute. However, when the apparatus was adjusted to drive the heart at 86 beats per minute the coronary flow was not augmented but slightly decreased (fig. 4A). In another series of observations the initial induced rate was 71 per minute and the coronary flow was about 35 cc. per minute. On increasing the rate to 86 the coronary blood flow increased to about 40 cc. per minute but an additional increment in the rate of stimulation of the heart did not increase the coronary blood flow since the heart did not beat at the increased rate. Successive decrements in heart rate of 15, 28 and 54 decreased the coronary flow from a control of 50 cc. to less than 20 cc. per minute (fig. 5). In another series of observations the heart was driven at 71, 58 and 32 beats per minute and the resulting coronary blood flow was approximately 54, 46 and 28 cc. per minute respectively (fig. 6).

In one experiment, the temperature of the perfusing blood was increased from 35.5°C. to 41°C. The heart rate accelerated from 120 to 154 beats per minute. The mean arterial pressure was kept constant. Coronary flow increased from 40 cc. to 67 cc. per minute.

The augmentation of coronary flow during acceleration of the heart rate cannot be easily explained. The controlled blood pressure during stimulation remained relatively constant. The coronary flow was sometimes increased while the uncontrolled blood pressure was decreased during the acceleration of the heart. Therefore, our results cannot be accounted for on the basis of changes of blood pressure.

The possible rôle of metabolites has been considered. We have shown that increasing the cardiac output as much as 275 per cent did not appreciably alter the coronary flow. Since blood pressure was kept constant the work of the heart was proportionately increased when the cardiac output was augmented. Under these conditions an increase in metabolites sufficient to affect the coronary vessels did not apparently occur. This being true one hesitates to advance the hypothesis that metabolites were responsible for the increased coronary flow accompanying the acceleration of the heart rate in view of the fact that the cardiac output was reduced and therefore the work of the heart was decreased in nearly all of the experiments in which the heart was driven at an increased rate.

The increase in coronary flow might conceivably have resulted from incidental stimulation of the cardiac nerves with a consequent vasodilatation of the coronary vessels. Attempts to eliminate the possible influence of the sympathetic nerves by injections of ergotoxine failed, as successive large doses of this drug did not prevent stimulation of the cardiac branches of the stellate ganglion from exerting its usual effect.

As already stated, stimulation of the sympathetic nerves was very effective in augmenting coronary flow when done early but ineffective when done late in an experiment. Since induced acceleration of the heart rate caused increases in coronary flow without respect to the life of the heart-lung preparation, the likelihood is not great that the sympathetic nerves were responsible for the increased coronary blood flow resulting from driving the heart at induced accelerated rates.

The presence of chemically active substances resulting from stimulation of the heart cannot be ignored as a possible factor in the increased coronary flow. An increased massaging action of the heart muscle during acceleration as stressed by Porter and Langendorff might be suggested as a factor in producing the augmented coronary flow but according to Wiggers this influence is of minor importance. That a decrease in the effective peripheral resistance of the coronary vessels occurred cannot be doubted but the factor or factors responsible for it are not apparent.

SUMMARY

In the heart-lung preparation the influence of various factors on coronary flow was studied with the thermostromuhr. In agreement with others an intimate dependence of coronary flow on mean arterial blood

pressure was found. Changes in cardiac output had no effect on coronary flow, if the arterial pressure and the temperature of the perfusing medium were kept constant. Stimulation of the cardiac branches of the stellate ganglion of the heart-lung preparation augmented coronary flow on an average of about 150 per cent. Similar values for coronary flow were obtained in the presence of a constant arterial blood pressure. The increase in coronary flow resulting from stimulation of the sympathetic nerves of the heart-lung preparation is thought to be due at least in part to a vasodilator action of the sympathetic nerves. An augmentation of coronary flow was demonstrated with acceleration of the heart rate by means of an electric stimulator. The augmented coronary flow was not due to an elevated mean blood pressure.

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THE CARDIAC OUTPUT IN REST AND WORK IN HUMID HEAT

ERLING ASMUSSEN

From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston

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In humid, hot climates evaporation of sweat on the surface of the body is greatly impaired, and the flow of blood through the skin must be increased in order to maintain a normal body temperature. The effect of this changed distribution of the blood on the circulation and its regulation has great physiological interest and only few investigations in this field are available (e.g., Dill, Edwards, Bauer and Levenson, 1931; Scott, Bazett and Mackie, 1940). A new opportunity for studying this question came when the staff of the Harvard Fatigue Laboratory in the summer of 1939 went to Benoit, Mississippi, in order to make physiological studies under the climatic conditions present in that part of the world. When our data were being collected in Benoit, the mean temperatures were 30.6°C. dry bulb and 27.6°C. wet bulb. Supplementary data for comparison were collected mostly during the following winter in Boston.

The subjects were all members of the laboratory staff except for a Negro sharecropper (B. C.), studied in Mississippi, who came to Boston as a laboratory helper in October. E. A. reached Benoit directly from Denmark about ten days before the determinations were started, whereas the other subjects at the time of the determinations (July, 1939) had been in Benoit for about one and one-half months. The estimations of the cardiac output in rest and work were made by the Grollman acetylene method, which gives the arterio-venous O_2-difference; this divided into the metabolic rate gives the cardiac output per minute. The metabolisms were determined from the ventilation and analyses of the expired air. Experiments in rest and during work will be treated separately.

a. Rest. For our resting experiments the subjects came in fasting in the morning and were asked to lie down to a prone position (or to sit down with legs horizontal) until the pulse rate had become stationary (usually after about 30 min). At least two determinations of the cardiac output were made on each subject.

Table 1 (A) gives the results from Benoit. The values presented are averages of all the single determinations. Table 1 (B) gives corresponding data from experiments carried out in Boston during the following winter on four of the subjects, and further from determinations on E. A. and M. N.

during the preceding winter in Copenhagen, Denmark. These experiments were all made at ordinary room temperature (22°C.). No winter values were obtainable on S. R. It is quite evident from the data that the cardiac output per m^2. of body surface (the cardiac index of Grollman) is the same in Boston as in Benoit. The average value in Benoit is 2.44 l. per min. and per m^2., in Boston, 2.49 (values for E. A. from Copenhagen not included). The individual changes are negligible except in the case

TABLE 1

Observations made in the resting state

	WEIGHT	SURFACE AREA	B.M.R.	A-V O_2 DIFF.	CARDIAC OUTPUT	CARD. OUTPUT / SURFACE AREA	PULSE	STROKE VOL.
			(A) In Benoit					
	kgm.	*m².*	*cc. O₂/ min.*	*cc./l.*	*l./min.*	*"card. index"*		*cc.*
E. A.	69	1.82	236	53	4.5	2.47	75	60
B. C.*	61	1.69	229	53	4.3	2.55	64	67
D. B. D.	76	1.95	225	51	4.4	2.26	56	79
M. N.	73	1.97	233	51	4.6	2.34	66	70
S. R.	61	1.70	220	52	4.2	2.47	74	57
J. W. W.	69	1.87	234	50	4.7	2.52	57	82
Average						2.44		
			(B) In Boston and Copenhagen					
E. A.	70	1.83	249	46	5.4	2.95	57	95
B. C.*	67	1.78	254	56	4.5	2.53	55	82
D. B. D.	79	1.98	241	55	4.4	2.22	56	79
M. N.†	83	2.09	249	52	4.8	2.30	55	87
J. W. W.	70	1.88	235	50	4.7	2.50	60	79
Average						2.49		
E. A.†	70	1.83	254	48	5.3	2.90	59	90

* Colored.

† Copenhagen.

of E. A., who shows a decrease from 2.95 in Boston (2.90 in Copenhagen one year earlier) to 2.47 in Benoit. The pulse rates in Benoit were, in the cases here described, slightly higher than in Boston or in Copenhagen. Accordingly the stroke volume was lower in Benoit than in Boston.

There seemed to be a correlation between the external temperature at the moment of determination and the pulse rate, which is shown for the subject E. A. in table 2.

The practically unaltered cardiac index found in our subjects when ac-

climatized to hot, humid climate indicates that a regulation has taken place. In acute experiments, lasting only for one to two hours, a considerable shift of blood from the central vessels to the periphery usually is accompanied by a decreased cardiac output, pulse acceleration, and vasoconstriction, impairing the functions of organs as the intestine and the kidneys (Asmussen, Christensen and Nielsen, III, 1939). It seems likely that for short exposures to high temperatures similar changes will take place, but that a prolonged exposure must demand a different regulation, allowing all organs an adequate blood supply. Facts supporting this assertion are presented by Scott, Bazett and Mackie, 1940, who found that the cardiac output in their subjects was slightly increased on the first few days of exposure to heat; the original values were then regained. At the same time an increase in blood volume had taken place. Our subjects had all been exposed to the heat for at least ten days and therefore are comparable to the subjects of Scott, Bazett and Mackie in the later stages of acclimatization. It is, therefore, reasonable to assume, as these authors do, that

TABLE 2

Cardiac output and heart rate of subject E. A. at Benoit

SHADE TEMPERATURE	SUBJECT'S LOCATION	NUMBER OF DETERMINATIONS	PULSE RATE	CARDIAC OUTPUT
°C.				*l./min.*
29	Shade	1	70	4.6
30	Shade	2	74	4.5
32	Sun	2	81	4.5

the normal cardiac index found in fully acclimatized individuals is made possible by the increased blood volume. The subjects of Scott, Bazett and Mackie showed increases of as much as 25 per cent or more in blood volume during their stay in a hot room; of our six subjects, four had a slightly increased blood volume in Benoit, one (D. B. D.) showed no change, and one (E. A.) even showed a slight decrease. (See Forbes, Dill and Hall, 1940.) The fact that this subject alone had a lower cardiac index in Benoit than in cooler climates seems to indicate that an increased blood volume is an important factor in maintaining a normal cardiac output.

Small daily variations in the amount of blood pooled in the skin apparently were compensated for by changes in the pulse rate, as table 2 shows. A high pulse rate can, as pointed out by Asmussen, Christensen and Nielsen (I, 1939), compensate for a decreased filling of the great central veins.

Recapitulating, it seems reasonable to assert that after acclimatization to a hot, humid climate has taken place, the cardiac output in rest is the

same as in a more temperate climate, and that compensation for the increased skin circulation is accomplished by means of an increased blood volume and probably by a slightly raised pulse rate.

b. Work. The work consisted in walking on a treadmill at a speed of 5.6 km. per hr. at an elevation of 8.6 per cent. This grade of work could easily be maintained for two to three hours in Boston's temperate climate, whereas it was very difficult in Benoit and for some subjects impossible to continue the prescribed two hours. The difference can, perhaps, best be described by stating that whereas a "steady state" could easily be

TABLE 3

Observations made during first one-half hour of walk

	O_2-INTAKE	A-V O_2 DIFF.	CARDIAC OUTPUT	CARDIAC OUTPUT / BODY WEIGHT	PULSE	STROKE VOL.
(A) In Benoit						
	l./min.	*cc./l.*	*l./min.*	*l./kgm.*		*cc.*
E. A.	1.60	108	14.8	0.21	141	105
B. C.*	1.47	117	12.6	0.21	132	96
D. B. D.	1.78	128	13.9	0.18	126	110
M. N.	1.79	120	14.9	0.20	146	102
S. R.	1.51	114	13.2	0.22	130	102
J. W. W.	1.81	111	16.3	0.24	137	119
Average		116	14.3	0.21		106
(B) In Boston						
E. A.	1.86	101	18.4	0.26	120	153
B. C.*	1.63	113	14.4	0.22	140	103
D. B. D.	1.93	120	16.1	0.20	132	122
J. W. W.	1.63	117	13.9	0.20	120	106
Average		113	15.7	0.22		121

* Colored.

reached in Boston, the pulse rate and the body temperature of some subjects in Benoit were steadily increasing, eventually making continuance of work impossible. For comparison between the circulation rate at a given rate of work in Boston and in Benoit, we have chosen the first half-hour of work, where a relatively steady state is reached, before a failure of circulation or temperature regulation is evident, and the last half-hour of the work, where in Benoit signs of a circulatory failure were apparent.

Table 3 (A and B) shows the results for the first half-hour of work. It will be seen that the average arterio-venous O_2-difference for the whole group is practically the same in Boston as in Benoit. The small difference

in cardiac output is mainly due to the difference in oxygen intake during the work, which was lower in Benoit owing to the fact that all of these subjects lost weight during their stay there. A comparable expression for the cardiac output in this kind of work is the cardiac output per kilogram of body weight, and table 3 shows that this is practically unchanged. Although there are great individual differences, the pulse rate for the whole group is somewhat higher in Benoit than in Boston and the stroke volume somewhat lower.

TABLE 4

Observations made during last one-half hour of walk

	O_2 INTAKE	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE	STROKE VOL.	DURATION OF WORK
			(A) In Benoit			
	l./min.	*cc./l.*	*l./min.*		*cc.*	*min.*
E. A.	1.82†	140	13.0	160	81	175
B. C.*	1.50	119	12.6	139	91	75
D. B. D.	1.78	131	13.6	138	99	75
M. N.	1.97	141	14.0	167	84	105
S. R.	1.56	111	14.1	162	87	120
J. W. W.	1.95	142	13.8	170	81	85
Average		131	13.5		85 ($\Delta = -19.8\%$)	
			(B) In Boston			
E. A.	1.96	111	17.6	120	147	180
B. C.*	1.66	116	14.3	140	102	120
D. B. D.	2.04	128	15.9	140	114	120
J. W. W.	1.67	126	13.3	135	99	120
Average		120	15.3		116 ($\Delta = -4.1\%$)	

* Colored.

† Extrapolated.

As the work progresses, however, the influence of the hot climate is more marked. Table 4 (A and B) represents values obtained during the last half-hour of the work. The last column shows the time at which the work was interrupted, either deliberately or, in some cases, because of exhaustion. It will be seen that the arterio-venous O_2-difference, both in Boston and in Benoit, is higher at the end than at the start but that the more marked increase is in Benoit. The cardiac output is slightly decreased, more so in Benoit than in Boston. The greatest effect can be seen in the pulse rate and the stroke volume. Whereas the latter in Boston is only decreased by about 4 per cent, it is decreased by 20 per cent in Benoit.

The demands of the hot, humid climate on the cardiac output during work seem, according to our results, to have been practically compensated for in acclimatized subjects. There is good reason to believe that the compensation has been accomplished in the same way as in rest, i.e., by an increased blood volume and an increased pulse rate. In prolonged work this compensation seems to be incomplete. The steadily increasing body temperature in the less fit subjects indicates rising difficulties for heat dissipation. An increased skin circulation will impair the filling of the central veins and of the heart. A fall in cardiac output and blood pressure will call forth a reflected rise in the pulse rate. If this fails to maintain an adequate cardiac output, the blood pressure will drop to levels where continuance of work will be impossible. It is, however, not possible to say whether the high body temperatures reached in these experiments or the inadequate blood supply to muscles and brain are the direct cause of the ultimate exhaustion. That the organism has thrown in all its reserves of blood is made evident by the fact that some of the subjects vomited or suffered from water-diarrhea immediately after the work, indicating that the blood supply to the gastro-intestinal organs was too low to allow them to absorb water drunk during or after the work. (Compare Asmussen, Christensen and Nielsen, III, 1939.)

The above results are in agreement with the results of Dill, Edwards, Bauer and Levenson, 1931, who by acute exposure to high or low temperatures during work found the same effects on pulse rate and stroke volume as we have found and showed that the systolic blood pressure during work was lower in the hot room than in a cold one, indicating possibly a failing blood pressure regulation.

SUMMARY

Acclimatization to humid heat seems to involve such regulations that the circulation in rest and during work in a steady state can be kept at a practically normal level. A blood volume increased by about 5 per cent (see Forbes, Dill and Hall) and a slightly higher pulse rate are assumed to be the two main factors in this regulation. Circulatory failure during work develops rather fast in humid heat owing to the fact that the heat dissipation is made difficult. A larger amount of blood is demanded for the skin circulation, making maintenance of an adequate cardiac output increasingly difficult.

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THE RELATION OF FASTING EXTERNAL PANCREATIC SECRETION TO HUNGER[1]

V. BROWN SCOTT, C. C. SCOTT AND H. J. BUGEL

From the Department of Physiology, Indiana University School of Medicine, Bloomington

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That a close relationship exists between the movements of the empty stomach and external pancreatic secretion was demonstrated by the extensive researches of W. N. Boldyreff (1), which amplified the reports of "periodic work" of the digestive canal by Russian investigators (Bruno, Schirokich, Tscheschkow, Klodnizki, Kaznelson, Edelman (2)). Boldyreff demonstrated that periods of motor activity of the empty stomach were accompanied by the secretion of intestinal juice, pancreatic juice and bile. These periods lasted 20 to 30 minutes and were followed by "rest" periods of 1½ to 2½ hours in which these organs were inactive, no motility or secretion being recorded. On this basis, Boldyreff postulated periodic "work" and "rest" for these organs in the fasting dog. Babkin (3) confirmed Boldyreff's conclusions but his experiments showed some pancreatic secretion during "rest" periods.

Since these early researches few confirmatory experiments have been conducted. E. B. Boldyreff (4) confirmed W. N. Boldyreff but concluded that concomitant gastric motility and pancreatic secretion were accompanied by a fall in blood-glucose of 20 to 30 mgm. per cent during the "work" periods. Zucker, Newburger and Berg (5) denied the existence of "periodic" pancreatic secretion, in animals having "total" drainage of the pancreatic juice by the method of Elman and McCaughan (6); these investigators reported a "continuous" fasting pancreatic secretion, not a "periodic" one.

Early in our investigations (7) we observed highly irregular pancreatic outflow in fasting dogs with fistulae of the Dragstedt (8) and Inlow (9) types. In the former fistula the flow was "continuous" with irregular periods of augmentation, while in the latter the pancreatic flow was irregularly periodic. Irregularities in the drop-by-drop pancreatic secretion were noted in both types of fistulae (Scott, 10). These irregularities in the rate-of-flow of pancreatic juice and appearance of periods of augmen-

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tation aroused doubts concerning the extreme regularity of the "periodic work" as reported by Boldyreff.

In previous experiments the type of gastric motility was not analysed and poor recording methods were used. It seems probable, however, that these investigators were inadequately recording low-grade gastric hunger contractions (Carlson, 11). Because of our experience in preparing many pancreatic fistulae (over 200) it was decided to re-investigate this problem employing better methods.

METHODS. Inlow pancreatic fistulae, as modified by us (12), were prepared in vigorous dogs. In this fistula the major pancreatic duct was transplanted to the abdominal skin (the accessory ducts were undisturbed) which permitted easy cannulation at each experimental period. Animals were gastrostomized according to the method of Carlson (13). This gastrostomy did not leak and proved superior to the metal-cannula method. Following recovery from the pancreatic fistula and gastrostomy each animal was trained to lie quietly, 24 hours post cibum, on a padded table. Pancreatic juice was collected by a glass cannula, cemented into the transplanted duct with collodion, connected to an automatic drop counter and recorder. Intragastric pressure was recorded by a water manometer connected to a condom balloon which was inflated to a constant pressure of 5 cm. of water and introduced into the fundus of the stomach via the gastrostomy; a spring device inside the balloon (14) maintained the fundic position of the balloon and simplified its introduction. Fluid gastric content, when present, flowed out of a separate intragastric tube.

Observations were conducted for 2 to 6 hours on the trained animals which appeared comfortable and quiet throughout the experiment; these dogs often slept during the greatest part of the experiment. When a dog's health failed because of prolonged loss of pancreatic juice, the animal was sacrificed. All of these animals lost weight, some losing as much as 30 per cent of their preoperative weight.

RESULTS. Ninety records of intragastric pressure and pancreatic secretion were obtained on 9 dogs. These records are summarized in table 1.

Records of group A were included because it was found that measurable quantities of pancreatic juice failed to flow from some fistulae, for periods often lasting for several days, followed by reappearance of pancreatic juice. No explanation has been found for this phenomenon, which seems to be a characteristic of this fistula. Thirty-two records, groups B and C, showed independent activity of the stomach or pancreas. Forty-eight records in group D evidenced both pancreatic secretion and hunger contractions with a high temporal correlation between them. Table 2 shows an analysis of these records.

Forty of these (83 per cent) showed hunger contractions and simultaneous augmentation of pancreatic secretion. Typical records will be noted

in figure 1. Eight records (17 per cent) revealed no apparent relationship between hunger and pancreatic secretion (see fig. 2). In these records hunger and secretion did not show a correlation, hunger appearing without secretion and vice versa.

The exact correlation between the peak-response of pancreatic secretion and the hunger period is shown in table 3, the hunger period being arbitrarily divided into three equal parts. It will be noted that pancreatic juice flowed most rapidly in the first two parts of the hunger period. However, rapid secretion was noted in the third part of the hunger period in a fair percentage of cases.

TABLE 1

Pancreatic secretion and hunger contractions in nine dogs having pancreatic fistulae and gastrostomies

GROUP	NUMBER OF RECORDS	RECORDS SHOWING PANCREATIC SECRETION	RECORDS SHOWING HUNGER CONTRACTIONS
A	10	0	0
B	24	24	0
C	8	0	8
D	48	48	48
Totals	90	72	56

TABLE 2

Analysis of records showing both pancreatic secretion and hunger contractions

GROUP	NUMBER OF RECORDS	CORRELATION BETWEEN HUNGER AND SECRETION	NO CORRELATION BETWEEN HUNGER AND SECRETION
D	48	40	8

Some records (table 1) exhibited a flow of pancreatic juice without an accompanying hunger period. These records were of interest because the rapid secretion often continued over a period of hours (fig. 3). Hunger contractions without accompanying pancreatic juice, seen in some records, was not attributable to accidental obstruction of the flow of juice because in all instances the cannula was examined to rule out such a possibility. We are certain that when pancreatic juice flowed from the duct, that flow was recorded accurately.

Discussion. The foregoing results show clearly that our experiments did not reveal the close relationship between gastric hunger periods and external pancreatic secretion claimed by Boldyreff. Of eighty records showing either pancreatic secretion or hunger or both, exactly fifty per cent revealed simultaneous periodic activity of these two organs.

In addition, we have shown that copious pancreatic secretion under fasting conditions can occur in the complete absence of gastric motility

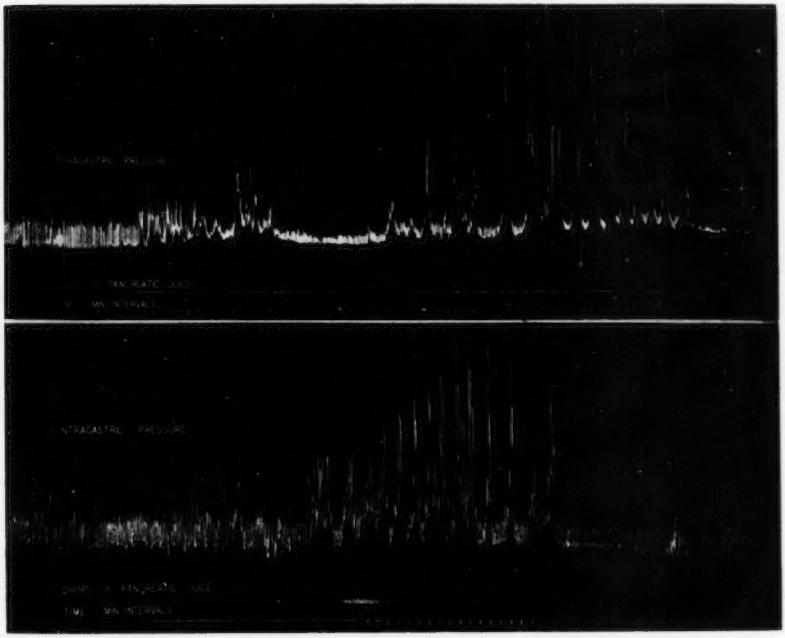

Fig. 1. Pancreatic secretion and gastric hunger contractions which show a temporal correlation.

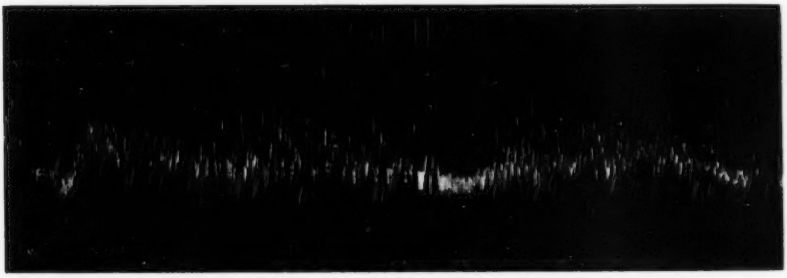

Fig. 2. Record of pancreatic secretion and intragastric pressure which shows no correlation between the activity of the stomach and pancreas.

(27 per cent of the records). Also, in nine per cent of the experiments, no pancreatic juice was secreted but strong hunger occurred. Boldyreff

reported that the periodic activity of one organ was always accompanied by simultaneous activity of the other digestive organs studied. This was not true in our animals, even though the periodic activity of any one organ was very strong. Furthermore, nine per cent of all records showed no apparent correlation between pancreatic secretion and hunger motility although the periodic activity of both organs occurred during the experiment.

These results are significant in view of Boldyreff's reports that the periodic activity was exceptionally regular. He stated that he could outline his program for an entire day's work, performing other work according to clock schedule during the "rest" periods between the periodic activity of the digestive organs. We have never observed such regularity of the

TABLE 3

Relation of the peak-response of pancreatic secretion to the hunger period

PART OF HUNGER PERIOD	NUMBER OF RECORDS
First	15
Middle	14
Last	6
Indeterminable	5

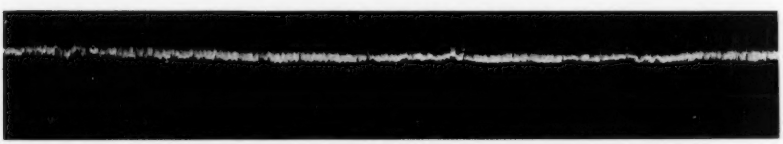

Fig. 3. Rapid pancreatic secretion unaccompanied by gastric hunger contractions.

periodic activity. An attempt to classify data according to the length of the active periods and the periods of rest was completely unsuccessful because pancreatic secretion and gastric motility were absolutely irregular, even in the same animal on the same day. Indeed, pancreatic secretion was often almost continuous. We can offer no explanation for the variation between our results and those of Boldyreff except that his animals had combined pancreatic and intestinal fistulae. It is possible that Boldyreff missed or ignored small amounts of pancreatic juice because of the type of fistula used.

From table 3 it appeared that the peak-response of pancreatic secretion and a particular part of the hunger period did not bear any constant relationship. It is true that the greatest volume-flow of pancreatic juice occurred most frequently in the earlier parts of the hunger period, but it was not uncommon for the peak-response to occur in the terminal portion

of this period. Just why there should be such variation is unknown and may be of no particular significance. The analysis is of interest, however, because it shows that activity of one organ does not invariably call forth the activity of the other immediately. This observation does not strengthen the belief that the activity of the two organs is controlled by a single mechanism.

Since one-half of our records (not including records in group A, table 1), showing pancreatic secretion and gastric motility, revealed a definite relationship between the two, it seems reasonable to believe that such observations are not pure coincidence; when both organs were active the correlation was 83 per cent. The mechanism responsible for this correlation has not been found.

Many possible explanations might be suggested but little concrete evidence has yet been advanced. E. B. Boldyreff (4) reported that during periods of fasting pancreatic secretion the blood-glucose level was lowered which was followed by an elevation of this level in the subsequent "rest" period. Additionally, he stated that prolonged stoppage of the pancreatic ducts produced mild diabetes mellitus. Mulinos (15) and W. W. Scott (16) reported no direct causal relation between hunger periods and the blood-glucose level. Dragstedt (8) found no evidence of diabetes mellitus in dogs with total pancreatic fistulae. Scott (17) has, in a few experiments, found inconstant blood-glucose fluctuations in dogs with pancreatic fistulae. Marked hypoglycemia following insulin is accompanied by an augmentation of pancreatic secretion and gastric motility. Some evidence (incomplete at present) indicates that the mechanisms of concomitant augmentation are not identical since vagotomy abolished the response of the latter to insulin without affecting the former. From our results it seems probable, therefore, that the relation between the pancreas and stomach is not the simple one postulated by Boldyreff.

Periodic pancreatic outflow is not the result of motility of the pancreatic ducts because the amount of this secretion usually exceeded the volume which could be held by the pancreatic ducts.

CONCLUSIONS

1. A temporal correlation exists between the periodic motility of the stomach (hunger) and the external secretion of the pancreas.

2. The correlation of this periodic activity was not always present since only 50 per cent of all records showed simultaneous activity of the stomach and pancreas.

3. When both fasting pancreatic secretion and hunger periods were present the correlation of this activity was 83 per cent.

4. Periodic activity of the stomach and pancreas occurred at irregular intervals and not with the regularity claimed by Boldyreff.

5. Fasting pancreatic secretion can occur in complete absence of hunger contractions and can fail to appear with the onset of powerful hunger contractions.

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THE EFFECT OF CHANGES IN THE CALCIUM CONTENT OF THE CEREBROSPINAL FLUID ON SPINAL REFLEX ACTIVITY IN THE DOG[1]

JEROME K. MERLIS

From the Department of Physiology, University of Louisville School of Medicine, Louisville, Kentucky

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Although the cerebrospinal fluid system has been the subject of extensive study, most workers have concerned themselves mainly with the mechanics of the formation and reabsorption of this fluid, relatively little attention having been paid to the relationships existing between the cerebrospinal fluid and the tissue fluid of the brain and spinal cord. The probability of free communication between the subarachnoid space and the tissue spaces of the nervous system has been demonstrated by Mott (1910), Weed (1914), and others. If such communication does exist, it is reasonable to assume that any change in the composition of the cerebrospinal fluid will be reflected by a similar change in the composition of the interstitial fluids, and thus possibly by modified activity of the neurones bathed by the latter.

Several workers have reported spontaneous neuromuscular activity following the intrathecal administration of various solutions. Collip (1920) observed tetanoid behavior in dogs following the introduction of small volumes of $NaHCO_3$, hypertonic NaCl and KCl, and Na_2HPO_4 into the lumbar subarachnoid space. $CaCl_2$ antagonized this motor activity, and it was concluded that the tetany was due to a disturbance in the concentrations of the various cations, especially of calcium. Huggins and Hastings (1933) observed that the injection of sodium citrate into the cisterna magna of the dog produced motor excitation which was antagonized by calcium chloride. Calcium citrate provoked no motor response. Mullin, Hastings, and Lees (1938) produced tetany by the cisternal injection of salt solutions which were ionically balanced except for the absence of calcium. Similar results were obtained with the injection of sodium citrate. Bathing the lower spinal cord with the calcium-free solution was ineffective, although here too citrate produced spontaneous neuromuscular activity.

[1] A portion of these data was presented as a preliminary report at the New Orleans meeting of the American Physiological Society in April, 1940.

METHODS. The action of low calcium solutions on the spinal cord has been reinvestigated using a method of continuous perfusion through the spinal subarachnoid space at constant pressure and temperature (Merlis and Lawson, 1939). Dogs were anesthetized with sodium barbital (0.25 gram/kgm. intravenously), the spinal cord was sectioned at T10, and the caudal segment of the cord was prepared for perfusion. As an indicator of the effect of changes in the calcium content of the perfusion fluid on the activity of simple functional neural units, the flexion reflex of the tibialis anticus muscle was elicited by stimulating the posterior tibial nerve at 5 to 8 second intervals with single shocks applied from a thyratron stimulator. The tension developed by the muscle was recorded on smoked paper by means of a torsion wire myograph.

The control perfusion fluid was an artificial cerebrospinal fluid of the following composition (moles/liter): Na—0.141, K—0.0033, Ca—0.00125, Mg—0.0012, Cl—0.152, HPO_4—0.00048, HCO_3—0.021, glucose—0.0034, urea—0.0022. A change in the calcium concentration was always compensated by an opposite change in NaCl concentration so as to have control and test solutions isosmolar. All solutions were brought to a pH about 7.4 with CO_2, using phenol red as the indicator.

The rate of flow through the spinal subarachnoid space was varied either by changing the perfusion head of pressure, or by maintaining a constant pressure head and partially obstructing the outflow by means of a constriction. Rates varying from 2 to 25 cc./minute with perfusion pressures of 8 mm. Hg or higher were used.

RESULTS. The subarachnoid perfusion of the balanced artificial cerebrospinal fluid had, in most cases, no effect on the flexion reflex. Occasionally a slight diminution of the reflex was apparent, but augmentation was never seen. When the calcium-free solution was substituted for the balanced solution, there was marked augmentation of the reflex, an increase in the tone of the muscle, and spontaneous twitching of the muscles of the lower half of the body (fig. 1). These effects usually appeared in from 1 to 5 minutes, at least a portion of the latency being accounted for by the 3 cc. dead space between the fluid reservoirs and the spinal subarachnoid space. Replacement by the balanced salt solution was followed by subsidence of these effects, usually within less than 5 minutes.

Sodium citrate (0.1–5.0 per cent) intrathecally was followed by similar motor activity, which was more intense than that seen with calcium-free perfusions.

Solutions containing an excess of calcium, in concentrations as high as four times the control value, had no demonstrable effect on the flexion reflex. Higher concentrations than these were not studied.

The effects of a calcium-free perfusion appeared to be more intimately connected with the rate of flow through the spinal subarachnoid space than

with the perfusion head of pressure. Successful perfusions were obtained with pressures as low as 8 mm. Hg and as high as 100 mm. Hg. The pressures were always kept well below mean carotid pressure to avoid the possibility of marked reduction in blood flow through the cord with consequent anoxic effects. With perfusion pressure constant, an increase in the rate of perfusion produced increased effects (fig. 2).

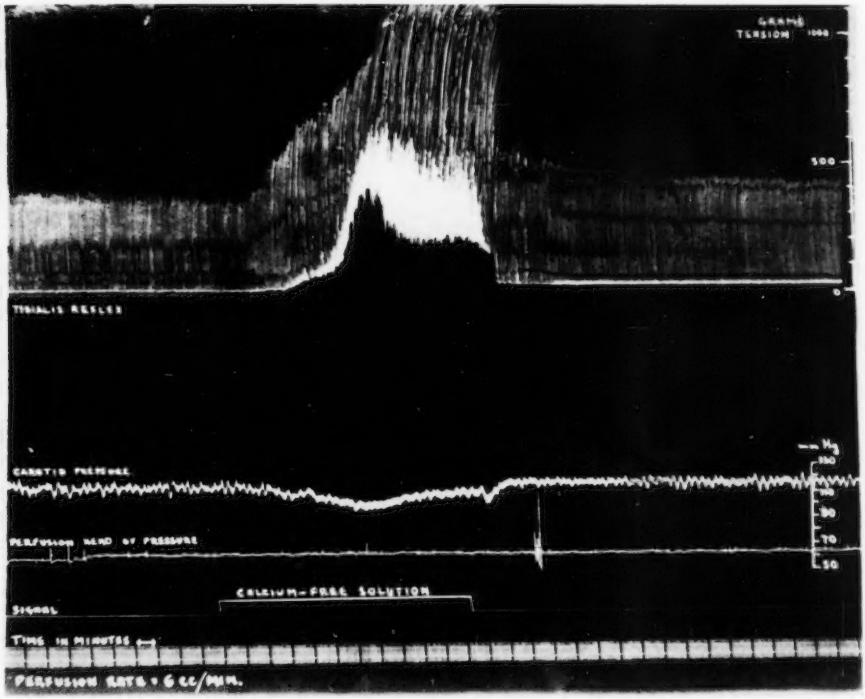

Fig. 1. The effect of a calcium-free perfusion. Irregular fibrillary contractions are recorded in the intervals between reflex contractions during the response.

Discussion. The failure of Mullin and his collaborators (1938) to produce tetany by bathing the spinal cord with low calcium solutions may be attributable to the method employed in their studies. If the action of these solutions is due to a lowering of the calcium content of the fluids bathing the cells of the central nervous system, it is essential that a diffusion gradient of sufficient magnitude be set up between the subarachnoid fluid and the interstitial fluids. Simple bathing of the exposed spinal cord with small volumes of salt solution might very well fail to achieve this end. The

effectiveness of the method of continuous subarachnoid perfusion may be ascribed to maintenance of an effective gradient.

It has been shown by Lehmann (1937) and by Brink and Bronk (1938) that a decrease in the calcium content of the fluids bathing peripheral nerve produces increased excitability of the nerve fibers and may provoke

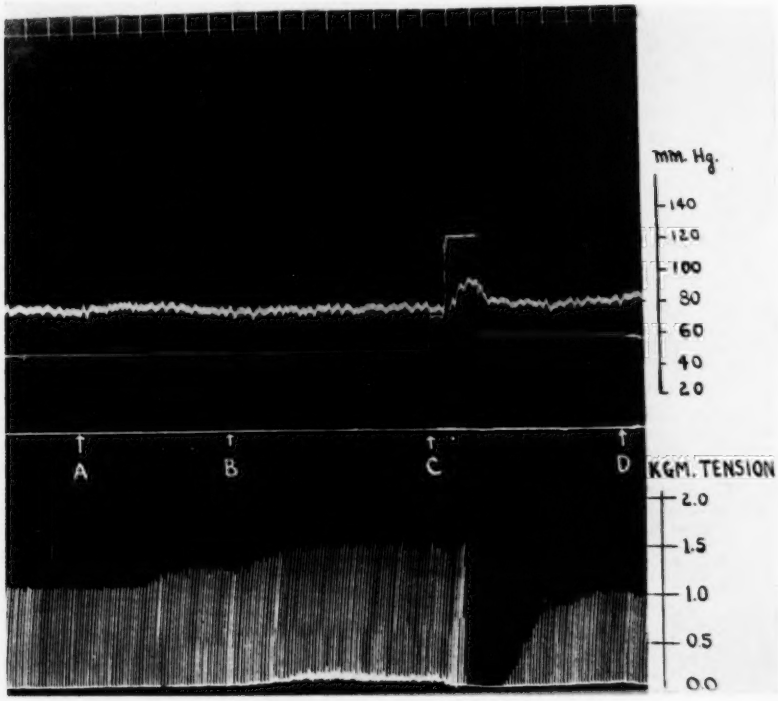

Fig. 2. Tracings from above downward: 1, time in minutes; 2, carotid pressure; 3, perfusion head of pressure; 4, signal; 5, tibialis anticus reflex. At *A*, calcium-free perfusion begun, perfusion rate: 3.4 cc./min. At *B*, perfusion rate increased to 16 cc./min. At *C*, perfusion pressure raised above carotid pressure. At *D*, calcium-free solution replaced by balanced salt solution. Fibrillary contractions are just visible in the intervals between reflex contractions during the Calcium-free perfusion, and disappear with the reflex during cord ischemia at *C*.

spontaneous discharge in many of them. The tetany produced by the spinal subarachnoid perfusion of calcium-free solutions might be due to an action of these fluids not in the cord itself but rather on the ventral rootlets in their intrathecal course. If this were true, synaptic transmision through the cord would not be essential, at least insofar as the spontaneous

twitching is concerned. This possibility has been tested by blocking synaptic transmission through the medium of the ischemia which is produced when perfusion pressure is raised above blood pressure (Luckhardt and Montgomery, 1929; Merlis and Lawson, 1939). When synaptic transmission was abolished by such a procedure, not only was the flexion reflex abolished, but so too were the spontaneous twitchings (fig. 2). It is therefore apparent that the muscular twitchings are not caused by spontaneous firing of the motoneurones of the cord, or of the nerve fibers of the anterior roots.

Two possibilities still remain: 1, the action is on the afferent limb of the reflex arc, i.e., spontaneous discharge of the dorsal roots or internuncial neurones, or 2, there is no spontaneous firing, but rather an increase in the excitability of the cord neurones, so that they respond more effectively to the normally incident flow of afferent impulses arising in the periphery. When the dorsal roots of the perfused cord segments were sectioned extradurally, spontaneous twitching was completely abolished, although synaptic transmission was still possible as was shown by eliciting reflex responses by mechanical stimulation of the central ends of the cut dorsal roots. This can mean only that the spontaneous tetanic manifestations depend upon the receipt of afferent impulses from the periphery and are not due to spontaneous firing of neurones of the cord, or of dorsal or ventral root fibers.

In the case of citrate perfusions, the situation is somewhat different. Ischemic blocking of synaptic transmission through the cord diminished, but did not abolish, the twitching which results from a citrate perfusion. Citrate, therefore, does cause spontaneous discharge of the motoneurones of the cord, or of the intrathecal ventral root fibers, or of both. Corroborative evidence was obtained from the deafferented preparation, where again there was diminution, but not abolition of twitching.

It is of interest to note the parallelism between the tetanic manifestations produced by calcium-free cerebrospinal fluid and those reported by West (1935) in his studies on parathyroid tetany. The results of his experiments led West to conclude that there were three neuromuscular manifestations of parathyroid tetany: fibrillary twitching, tonic, and clonic contractions. The clonic and tonic contractions were abolished by deafferentation of the cord, although the fibrillary movements were still in evidence after this operation. This finding is quite similar to that reported in this study, in which the tetany, although definitely central in origin, does depend upon the integrity of the somatic reflex arcs before it may be manifested.

SUMMARY AND CONCLUSIONS

1. The effect of changes in the calcium content of balanced salt solutions perfused through the lower spinal subarachnoid space at constant pressure

and temperature was studied in barbitalized dogs with spinal cord sectioned at T10.

2. Calcium-free solutions produced an augmentation of the spinal flexion reflex, an increase in muscle tone, and spontaneous twitching of the muscles of the lower half of the body.

3. The tetany produced by calcium-free perfusions is not due to spontaneous firing of the motoneurones nor of the dorsal or ventral rootlets. The twitching requires the integrity of the spinal reflex arcs, for it disappears when these arcs are broken. It appears to be due to an increased responsiveness of the cord neurones to the normally incident afferent impulses from the periphery.

4. Similar motor activity was seen with sodium citrate perfusions, differing in that the twitching persisted, with diminution, when synaptic transmission was abolished. Citrate appeared to cause spontaneous firing of the motoneurones, or of the dorsal and ventral rootlets, or both.

5. High calcium solutions, up to four times normal concentrations, were without effect.

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A SYNDROME OF POLYDIPSIA AND POLYURIA INDUCED IN NORMAL ANIMALS BY DESOXYCORTICOSTERONE ACETATE

CHARLES RAGAN, JOSEPH W. FERREBEE, P. PHYFE, DANA W. ATCHLEY AND ROBERT F. LOEB

From the Departments of Medicine and Neurology of the College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital and Neurological Institute, New York City

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Desoxycorticosterone has been shown to have a marked effect upon the renal excretion of sodium. The administration of this hormone to patients with Addison's disease or to dogs in uncompensated adrenal insufficiency results in a retention of sodium which under certain circumstances may be associated with a striking accumulation of extracellular fluid and symptoms of congestive heart failure (1, 2). We have not been able to produce these symptoms in normal animals. When normal dogs are given 25 mgm. of hormone in daily injections there is no striking accumulation of extracellular fluid and no congestive heart failure. Instead the normal animals develop a syndrome resembling that of diabetes insipidus, as might have been anticipated from the earlier work of Teel (3), Wermer (4), and also Silvette and Britton (5).

EXPERIMENTAL. The evolution of the syndrome can be seen in the accompanying chart of a normal female dog (fig. 1). This was an 11 kgm. animal kept in a metabolism cage, given one and one-quarter pounds of fresh raw lean meat each day, a haliver oil capsule, and all the water she wanted to drink. Throughout the experiment the urinary excretion of sodium, chloride, potassium, and nitrogen was measured in five-day periods. At the beginning of each period the blood serum was analyzed for sodium, potassium, and total protein. The data are presented in table 1 and figure 1.

For the two weeks before hormone injections were given, the animal's water intake averaged 400 cc. a day. The urine volume was also about 400 cc. a day with a specific gravity of 1.045. When subcutaneous injections of desoxycorticosterone acetate were begun, the effect upon the intake and output of fluid was striking. At the end of six weeks the dog was drinking not 400 cc. but 1000 cc. a day. A similar change occurred in the urine volume which also increased to about one liter a day with a specific gravity down to 1.025. These changes were not accompanied

by any evidence of excessive fluid retention or congestive heart failure. The balance studies (table 1) indicated that no great retention of sodium had occurred although the serum sodium concentration was consistently elevated. The serum protein concentration was essentially unaltered. The dog had gained slightly in weight probably because $1\frac{1}{4}$ pounds of meat a day was more than her maintenance requirement. This gain of weight had commenced before the injections of hormone were begun.

At the end of seven weeks, the meat was reduced to three-quarters of a pound a day, the injections were continued, and in addition to the hor-

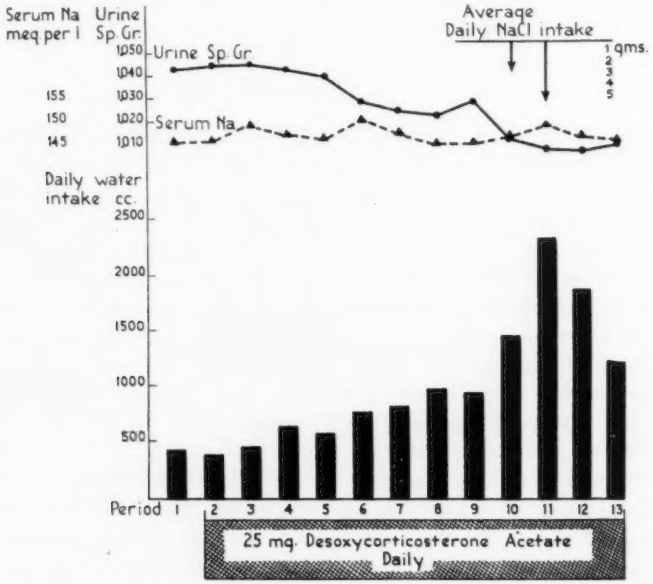

Fig. 1. Evolution of polydypsia in course of treatment of normal dog with desoxycorticosterone acetate.

mone the animal was given 40 to 80 m.eq. of sodium chloride a day in her drinking water. The addition of this amount of sodium chloride to the regime resulted in a further increase in water intake accompanied by an equal increase in urine output and a further decrease in urine specific gravity down to a level of about 1.008. After one week on the high salt regime the animal developed a peculiar muscular weakness which has been seen in all five of our dogs on similar regimes and has been previously described (6). The administration of sodium chloride was stopped and the volume of fluid exchange returned to about 1 liter a day.

The effect of potassium chloride was then studied. Thirty to 80 m.eq. of potassium chloride were administered to the animal of figure 1 each day for a period of ten days. No sustained effect upon water exchange was demonstrable although the specific gravity of the urine rose to 1.015. Potassium chloride was stopped, sodium chloride was again administered, and the usual effects of salt were observed: increase in fluid intake, increase in urine output, decrease in urine specific gravity.

In another dog we have studied the effect of pitressin upon this syndrome of polydipsia and polyuria. Figure 2 is the chart of a normal male ani-

TABLE 1

Balance studies of normal dog treated with desoxycorticosterone acetate

PERIOD	WEIGHT	AVERAGE DAILY INTAKE					AVERAGE DAILY OUTPUT						BLOOD AT END OF PERIOD					
													Serum				Plasma NPN	Blood sugar
		Meat	Desoxycorticosterone acetate	NaCl	KCl	Water intake	Urine volume	Sp.G.	N_2	Na	K	Cl	Na	K	Cl	Protein		
	kgm.	*lbs.*	*mgm.*	*m.eq.*	*m.eq.*	*cc.*	*cc.*		*grams*	*m.eq.*			*m.eq. per l.*			*grams*	*mgm. per cent*	
1	10.9	1.25	0	0	0	425	415	1043	15.5	6.5	43.5	4.6	145	4.4	107	6.7	31	77
2	11.3		25	0	0	395	365	1046	16.8	4.0	44.6	5.6	146	4.1	109	6.2	35	78
3	11.5					445	420	1047	16.5	6.0	46	7.2	149	3.9	107	6.5	29	77
4	11.7					615	485	1044	16.1	5.4	48	8.3	148	3.8	107	6.1	33	82
5	11.9					580	675	1041	16.5	5.4	48.2	9.1	147	3.3	105	6.1	33	77
6	12.0					760	805	1029	17.8	6.6	49	9.1	151	3.3	107	6.6	24	69
7	12.2					810	810	1026	17.7	5.9	43	6.8	148	3.2	104	6.6	29	74
8	12.4					975	1010	1023	17.7	11.5	44.8	9.6	146	3.1	103	6.5	28	80
9	12.4					925	940	1028	17.2	10.4	42.4	8.7	147	3.5	102	6.6	31	77
10	12.1	0.75	25	38.8	0	1490	1445	1014	10.6	43.3	34.2	45	147	3.3	107	6.3	23	74
11	12.0			85.6	0	2330	2250	1009	10.1	79.8	32.6	85	150	2.9	107	6.3	20	74*
12	11.8			0	0	1875	1800	1008	10.5	12.4	21.2	8.7	148	3.2	100	6.4	27	76
13	11.8			0	0	1280	1215	1011	11.0	9.0	23.8	5.6	147	3.2	101	6.7	23	80
14	11.8			0	33.8	1535	1490	1009	11.1	16.7	36.8	32.2	147	4.0	109	6.3	24	71
15	11.6			0	79.0	1420	1290	1014	9.9	13.9	95.3	79	148	4.1	111	6.5	27	111
16	11.5	0.75	25	81	0	1930	1835	1010	10.7	78.0	48.4	90.2	147	3.2	106	6.8	23	70
17	11.6			51	0	1720	1500	1010	10.0	40.9	25.0	46.0	147	2.4	108	5.9	20	72*
18	11.8			107	0	2910	2720	1006	10.7	90.0	35.5	102	152	2.7	115	6.4	28	85†

* Paralysis.

† Paralysis and death.

mal in which the syndrome had been well established by the administration of 8.5 grams of sodium chloride and 25 mgm. of hormone each day for several weeks.

Pitressin subcutaneously in rather large doses caused some decrease in the fluid exchange with a slight decrease in the serum sodium concentration and a slight increase in the urine specific gravity. Restriction of the animal's intake to two liters for one day resulted in a sharp rise in the serum sodium concentration and an increase in the urine specific gravity. Following this restriction, there seemed to be a compensatory increase

in water intake to 9000 cc. with a comparable increase in urine volume and a resultant drop in urine specific gravity and serum sodium concentration.

The injections of hormone were then stopped but the administration of sodium chloride was continued. Despite continuance of salt administration there was a prompt cessation of polydipsia and polyuria. In the course of seven days the urine specific gravity rose to normal, the water exchange fell from seven liters down to one and one-half liters a day, the serum sodium concentration remained about the same.

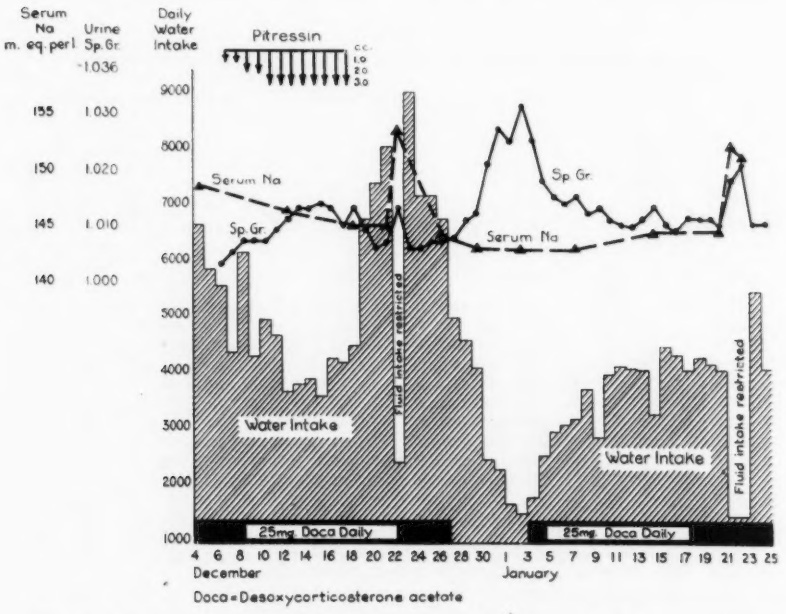

Fig. 2. Effects of pitressin, fluid restriction and withdrawal of desoxycorticosterone acetate upon the polydypsia produced in a normal dog by desoxycorticosterone acetate and sodium chloride.

The readministration of hormone resulted in reappearance of the entire syndrome with increased water consumption, increased urine volume, and decreased urine specific gravity.

When the syndrome was again well established, the intake of fluid was restricted for two days. Serum sodium concentration and urine specific gravity rose sharply and the animal was obviously thirsty and very uncomfortable although he did not go into negative fluid balance or show significant evidence of dehydration such as loss of weight or increased

serum protein concentration. When free access to fluid was again allowed, there was a sharp increase in fluid intake with a concomitant drop in serum sodium concentration and urine specific gravity.

Observations similar to those which have just been described were made upon three other normal dogs, two females and one male. The male animal was killed with chloroform and autopsied when the symptoms of diabetes insipidus were well established. The adrenal glands of this animal were somewhat small but the other organs and the microscopic sections of the pituitary gland, kidneys, adrenals, and gonads were essentially normal. It is interesting that none of the normal animals developed symptoms of adrenal insufficiency when the injections were stopped.

We were not able to produce polyuria and polydipsia in normal rats on a low salt, low potassium diet, although enough hormone was used to produce an atrophy of the adrenal glands. In normal adult male rats kept on a high salt diet, we were able to double the water intake by giving the animals 5 mgm. of hormone daily. The hormone-treated animals drank 300 cc. of water a day and had an average urine specific gravity of 1.015. The control rats that were given a high salt diet and no hormone drank 150 cc. of water a day and had a urine specific gravity of 1.022.

Discussion. It is tempting to speculate upon the mechanism by which desoxycorticosterone acetate produces polydipsia and polyuria in normal animals. Although the syndrome superficially resembles that of diabetes insipidus the two states are dissimilar in at least two important respects: pituitrin is relatively ineffective and fluid restriction does not cause dehydration in our animals. The converse is true in diabetes insipidus. It seems probable, therefore, that the condition which we have described is primarily a thirst and only secondarily a polyuria. The cause of the thirst is not clear, but it may be related to the maintained increase in extracellular sodium concentration or to some associated disturbance of the osmolar balance between extracellular and intracellular fluid. Whatever the mechanism of the polydipsia may be, the consequent polyuria enables the normal dog to avoid excessive retention of sodium. A rapid flow of urine through the tubules would tend to counteract the increased sodium reabsorption brought about by the adrenal cortical hormone.

CONCLUSIONS

Large doses of desoxycorticosterone acetate induce in normal animals a syndrome of polydipsia and polyuria similar to that seen in diabetes insipidus.

The mechanism of this effect and its differentiation from diabetes insipidus are discussed.

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HEAT EXCHANGE AND REGULATION IN RADIANT ENVIRONMENTS ABOVE AND BELOW AIR TEMPERATURE

C.-E. A. WINSLOW, A. P. GAGGE AND L. P. HERRINGTON

Contribution no. 29, from the John B. Pierce Laboratory of Hygiene, New Haven, Conn.

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Object of study. In previous contributions from this laboratory (Herrington, Winslow and Gagge, 1937; Winslow, Herrington and Gagge, 1938) data have been presented with respect to the reactions of the human body to atmospheric conditions in which air and wall temperatures were independently varied. Our results suggested that a cold-air warm-wall situation exhibited unexpected phenomena, the skin temperature of the subjects being lower than in the case of exposure to equal wall and air temperatures having, physically, an equivalent cooling effect.

In these earlier experiments, our high radiation was produced by reflection from the surfaces of a copper booth in which the subject under observation was placed; and we had no means of studying the influence of cold walls with warm air. Also, air movement was not recorded in each experiment but assumed from preliminary tests. With the construction of a new experimental booth designed on a different principle, it has been possible to study both the cold-air hot-wall situation and the hot-air cold-wall situation under comparable conditions and to pay special attention to air movement.

Description of the new experimental booth. The new booth is octagonal in shape, each of its eight walls being four feet wide and eight feet high and the distance across the booth between the centers of each pair of opposite panels being ten feet. Each panel consists of a steel frame supporting a four by six foot copper tank of one inch interior thickness through which may be circulated a "brine" solution of di-ethylene glycol and water. The temperature of the panels can be reduced to −6°C. by passing the solution through an ammonia brine cooler or can be raised to 72°C. by passing the solution through a gas-fired water-heating boiler. Alternate panels are hinged so that they can be swung outward, and the "brine" solution is supplied to these movable panels by short lengths of rubber hose. The interior face of the panels is painted black for maximum emissivity from the radiant surfaces, and the floor and ceiling of the booth are of aluminum to increase reflectivity from those surfaces which are not directly heated or cooled.

The booth is placed in the center of a seventeen-foot square shell of steel, and this shell, in turn, is inside a twenty-foot square room insulated with six inches of cork on all six surfaces. Conditioned air is introduced from a York conditioning unit which can supply air at any temperature between −18 and +60°C. with an accuracy of ±0.5°, and can control the relative humidity within 2 per cent over a dry-bulb range between 5° and 60°C., and within the limits of ten per cent and ninety per cent of saturation.

The conditioned air enters the insulated room near the ceiling and is directed by dampers into the steel shell, upward or downward, along all its four sides. The booth itself has dampers above and below the radiant panels and the air introduced into the steel shell passes upward or downward through the booth, depending on panel temperature. The actual air velocity within the booth can be controlled by the upper and lower dampers and is ordinarily maintained at a level of about seven centimeters per second.

Conduct of experiments. The new experiments here reported were all performed with the same two young male subjects (VII and IX) used in earlier experiments, unclothed (except for an athletic supporter) and in a semi-reclining position. At the beginning of an experiment, the subject is seated in a movable chair (with an aluminum frame and canvas back and seat). In this chair he remains for one hour in an equalizing chamber, maintained, in all cases, at a temperature between 30° and 31°C., with a relative humidity of 40 to 50 per cent and an air movement of approximately ten centimeters per second. After this preliminary treatment, which produces a reasonably standard thermal adaptation, the chair is wheeled into the booth and transferred by jacks to the platform scale used for measuring evaporation. The standard period of observation in the booth has been 240 minutes, in the major experiments here reported.

Air temperature and relative humidity within the booth are automatically recorded throughout. Mean radiant wall temperature is observed before the subject is brought in by the use of the Vernon globe thermometer placed in the center of the booth; and this measurement is correlated with the temperature of the brine within the panels, the latter temperature being recorded throughout the experiment. The brine temperature, which can be measured with high accuracy, is used in our final analysis for computing wall temperature on the basis of the established correlation between this reading and that of the globe thermometer. Air movement is measured by the hot-wire anemometer at the close of each experiment, with the chair present but without the subject, and at vertical levels corresponding to the position occupied by the head, chest, and calf of the subject when in the chair. The reasons for making this measurement without the presence of the subject have been outlined in a previous communication (Winslow, Gagge and Herrington, 1939).

During the course of an experiment, the following routine observations were made:

a. Rectal temperature, every 15 minutes, by a thermocouple inserted 14 cm. into the rectum and recorded on a potentiometer in the control room.

b. Skin temperature at 15 representative points on the body surface (Winslow, Herrington, and Gagge, 1936) measured every half hour by a Hardy thermopile held one centimeter from the body surface. Before and after each series of readings a calibrating observation was taken from two thermostatically controlled reference baths maintained at approximately 8°C. differential and including the skin temperature range observed.

c. Metabolism every 45 minutes by application of the Benedict-Roth apparatus. The subject breathed for the usual 6-minute interval in connection with an oxygen stream driven by motor-circulation through soda-lime and connected with a respirometer acting as an oxygen reservoir. The recording apparatus was in the control room.

d. Evaporative heat loss determined every half-hour by readings of weight loss on the platform scale upon which the subject sat.

Measurement of radiation area. Of the five factors in thermal interchange, metabolism and evaporation are, of course, observed directly. To estimate radiation interchange we must know not only the mean skin temperature of the subject and the mean radiation temperature of surrounding surfaces but also the radiation area of a given subject in a given position.

To determine this point we made a special series of experiments in which convection interchange was practically eliminated by holding air temperature approximately the same as skin temperature. Under such conditions, the algebraic sum of metabolism, evaporation, and storage should show a linear relation to the radiant heat interchange, as computed by the Stefan-Boltzmann Law from the observed mean skin temperature and the mean radiant wall temperature (Gagge, 1936).

The result of this relationship is indicated in figure 1. In computing the ordinate data we obtained storage values by assuming that change in total tissue temperature could be determined from mean change in skin temperature and rectal temperature between 40 and 200 minutes, giving the skin temperature a weight of one and rectal temperature a weight of two.

It will be seen from figure 1 that under neutral and warm conditions, the linearity law holds, and the line passes through the origin, thus validating the basic measurements involved. Under cold conditions, however, the linearity relation fails, indicating that our method of estimating storage (by a 1:2 weighting of skin and rectal temperature) was not justified. We shall return to this question in a later paragraph. Under extremely

hot conditions a deviation is again observed as a result of excessive sweat secretion, which drops off without cooling the body.

Where the linearity relationship does hold, the slope of the graph gives us the radiation area. For subject VII, this value is 1.47 sq. m. or 73 per cent of his DuBois area; for subject IX, it is 1.19 sq. m., or 74 per cent of his DuBois area.

In earlier studies (Gagge, Winslow and Herrington, 1938) we found a higher value for subject VII and a somewhat lower value for subject IX. The weight of subject VII changed materially during the interim and habitual posture maintained in the chair may also have influenced the

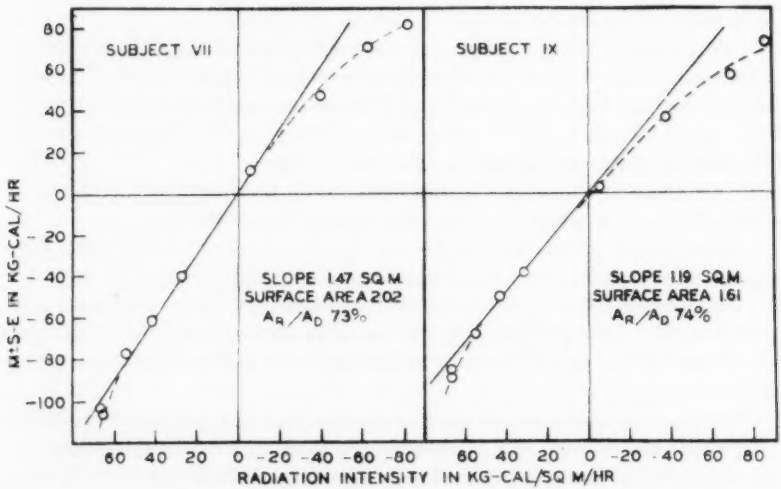

Fig. 1. Graph of heat exchange plotted against radiation intensity for determination of radiation area (with mean skin temperature equal to air temperature).

radiation area. We are inclined to believe that 70 to 75 per cent of the DuBois area is a representative value.

Determination of the convection constant. Having found the radiation area for each subject, our next problem was, of course, to determine the respective convection constants: and this has been done in a different way from that previously employed. In our earlier work, we computed K_C for a standard air movement of eight centimeters per second, which we assumed to hold approximately throughout the experiments. In the present series, the air movement was measured at the end of each experiment. We have shown elsewhere (Winslow, Gagge and Herrington, 1939) that air movement increases convective cooling in proportion to the square of its velocity (within the range of air velocities involved). With our new

data it is now possible to obtain a basic K_c for unit air velocity. For this purpose, we have again computed storage from mean changes in skin temperature and rectal temperature between 40 and 200 minutes, as before, weighting these two changes in the ratio of 1:2. We then (fig. 2) plotted convection loss ($M - E \pm R \pm S$) against the cooling effect represented by $\sqrt{V}\ (T_S - T_A)$.

The data represent four series of experiments, conducted, respectively, with warm-walls, neutral walls (equal to air), cool walls, and cold walls. For each series the interval between wall and air temperature was the same throughout, with varying air temperature, and, hence, varying calorie demand. As in figure 1, figure 2 shows that where the environ-

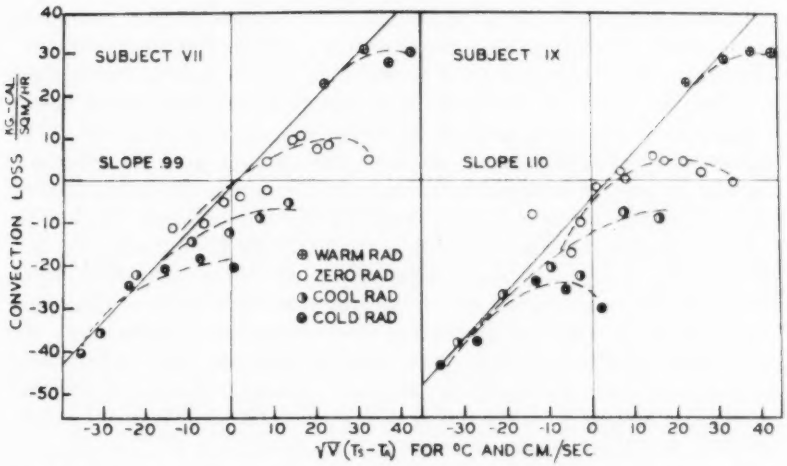

Fig. 2. Graph of convection loss plotted against product of air velocity and temperature differential for determination of convection constant.

ment is warm a common linear relationship is apparent and the line approximately intercepts the origin. From this line, we find that the K_c for unit air velocity is 0.99 for subject VII and 1.10 for subject IX.

In our earlier work with nude subjects we computed convection constants not per unit air movement but for standard air movement of eight centimeters per second. The values obtained (Gagge, Winslow and Herrington, 1938), when converted to unit area of subject and to unit air velocity are somewhat higher than those here reported. We have reason to believe—as will be indicated in a later paragraph—that the air velocities must have often exceeded the assumed value and the figures presented are, therefore, too high on a unit air velocity basis. Working with the present subjects (VII and IX), lightly clothed and with careful check on air move-

ment, we have reported a value of 1.04 per square meter of body surface as the basic convection constant, to be multiplied by the square root of air velocity to yield the total convection constant (Winslow, Gagge and Herrington, (1939). We believe that for both nude and clothed subjects in a semi-reclining posture the value of this basic constant is close to unity.

Storage values and skin temperature changes. It is obvious from the deviation of cold points in our curves from the straight lines of figures 1 and 2 that our estimates of storage for cold environments on the assumptions made above are much in error. We are confirmed, therefore, in our previous conviction that the only sound basis for estimating storage (outside the zone of linearity) is by difference—after accurate determinations of radiation and convection.

In the method of estimating storage from changes in skin and rectal temperatures, there are two possible pitfalls. In the first place, the question arises, from what point one should measure the rate of fall in these two temperatures. In the preliminary computations from which figures 1 and 2 were prepared, we took the mean rate of fall for successive 40-minute periods (40–80 min., 80–120 min., 120–160 min., and 160–200 min.). For the purpose of determining linearity relationships this—or any other rational assumption—would serve equally well. For determining absolute values, however, it seems erroneous to consider only the fall after the first 40 minutes, since, as we have shown (Winslow, Gagge and Herrington, 1939), the skin temperature falls very rapidly on initial exposure to a cold condition, as a result of immediate vasoconstriction. Storage, on the other hand, must be a much slower process, only catching up gradually with the primary physiological reaction. It seems, therefore, unsound to compare storage with the fall in skin temperature which occurs subsequent to the initial vasoconstriction.

In the second place, the computation of storage from changes in T_S and T_R involves a purely arbitrary assumption of the ratio to be assigned to each. It is obvious that this ratio must vary widely with the conditions of the experiment. In a slightly cool environment, only the very superficial areas are chilled; while under very cold conditions the area of tissue chilling must be deeper. It seems highly possible that in extreme circumstances, the bones and other less vascular parts of the body may be well below the temperature maintained in the blood stream which circulates so largely through organs such as the liver and brain where heat production is considerable.

From the results in table 1, we can make some estimate of what the actual significance of skin and rectal temperatures may be with respect to real storage. It will be noted that this table contains, in addition to columns for air temperature and wall temperature, a column for operative temperature. The figures in this column represent the combined cooling

effect of air and walls, as determined by the appropriate weighting of the physical effects of radiation and of convection, with the subjects concerned

TABLE 1*

Mean responses of two unclothed subjects to variations in air and wall temperature

CONDITION	T_A	V	T_W	T_O	T_S	T_R	M	E	R_H	C	CAL. S	Obs. S	K
	°C.	cm./sec.	°C.	°C.	°C.	°C.	kg. cal. per square meter per hour						kg.-cal./sq.m.-hr.-°C.
4	26.9	7.2	40.0	34.9	35.3	37.14	45.5	−42.2	20.4	−23.2	−0.6	−0.5	24.4
8	23.6	7.7	36.0	30.9	35.0	37.11	46.7	−21.2	4.3	−32.8	0	3.0	23.5
12	19.5	7.7	31.5	26.3	33.1	36.82	46.0	−15.6	−6.6	−39.0	2.2	15.2	16.4
16	15.9	7.6	28.4	23.2	31.7	36.51	47.3	−13.7	−13.3	−44.8	6.5	24.5	14.9
21	41.4	7.3	40.7	41.2	36.1	37.31	44.4	−70.9	20.1	14.8	−1.9	−8.4	29.8
31	38.5	5.3	38.1	38.1	35.9	37.34	45.8	−66.7	8.8	6.3	−1.9	+5.8	35.8
17	36.5	7.4	35.7	35.9	35.7	37.32	45.4	−52.1	0	2.3	−1.2	4.4	30.7
29	34.5	5.7	33.9	34.2	35.4	37.17	45.6	−44.1	−6.3	−2.1	−1.2	6.9	29.6
1	32.8	7.6	32.8	31.2	35.6	37.32	46.1	−29.4	−11.8	−8.1	−0.9	13.2	34.4
25	31.7	6.4	30.6	31.1	35.1	37.11	45.1	−23.4	−18.6	−8.9	−0.9	5.8	25.2
5	28.6	7.9	28.9	28.6	34.6	37.03	44.8	−12.7	−23.4	−17.4	0	8.7	22.0
35	27.5	5.6	26.7	27.4	33.9	36.98	47.6	−12.8	−29.0	−15.6	+1.5	9.8	18.6
9	25.0	8.6	24.9	24.6	33.6	36.78	44.2	−10.3	−36.0	−26.0	+3.1	28.1	22.7
39	23.8	6.7	22.9	23.7	32.2	36.91	48.7	−11.8	−36.5	−22.0	+3.4	21.6	14.9
13	21.7	10.8	21.3	20.0	31.9	36.81	45.1	−10.1	−40.1	−41.3	+5.6	46.4	18.5
23	47.8	6.8	35.6	40.4	35.9	37.34	47.0	−79.8	−1.3	31.9	−1.5	2.2	34.1
19	43.4	7.1	31.4	36.2	35.2	37.15	46.5	−53.8	−15.8	22.5	−1.5	0.6	24.2
3	38.9	7.6	28.0	32.4	35.5	37.25	44.4	−30.5	−30.7	9.7	−1.2	7.1	29.4
7	34.7	7.6	24.4	28.7	34.5	36.96	42.7	−18.8	−40.4	.5	0	16.0	23.8
11	31.2	8.4	21.2	25.5	33.7	37.01	46.6	−11.4	−49.0	−7.5	3.7	21.3	20.5
15	26.9	8.3	18.0	22.0	32.1	36.75	46.2	−9.8	−54.0	−15.4	6.5	33.0	17.0
18	48.7	7.0	26.9	35.7	35.2	37.08	46.0	−50.8	−33.7	37.1	0	1.4	25.2
2	44.7	7.6	24.0	32.6	35.3	37.33	43.8	−31.1	−45.3	26.9	−1.2	5.7	24.4
6	39.6	7.6	20.6	28.8	34.3	36.95	43.8	−14.3	−53.6	15.2	+0.9	7.9	19.5
10	35.4	8.7	17.8	25.7	33.3	37.00	44.2	−13.7	−59.7	6.5	+3.7	21.7	17.8
14	31.4	8.1	14.0	21.9	32.0	36.67	45.9	−14.2	−67.5	−1.8	+5.9	37.6	20.0

* T_A, ambient air temperature; V, air movement measured in booth with chair but without subject; T_W, mean radiant wall temperature; T_O, operative temperature including the combined effect of wall temperature, air temperature, and air movement; T_S, mean skin temperature; T_R, rectal temperature; M, the metabolism; E, evaporation loss; R, the radiation exchange; C, the convection exchange; Cal. S, storage calculated from change in skin and rectal temperature weighted 1:2, respectively; Obs.S, the algebraic sum of $M - E \pm R \pm C$; K, conductance, the ratio of $M + S$ to $T_R - T_S$.

and the wall temperature, air temperature and air movement which obtained. It is the temperature of air which—with equal wall and air

temperature and air velocity of 7 cm. per second—would exert the cooling effect actually produced. The table contains two columns for storage, one computed by weighting change of skin temperature and rectal temperature in the respective ratios of 1 and 2, the other as determined by the algebraic sum of $M - E \pm C \pm R$. It will be noted that at all operative temperatures below 40°C., true storage is higher than storage estimated from ΔT and that below an operative temperature of 26°C., the difference becomes very great, rising from 10 up to over 40 kilogram calories.

To test further this somewhat surprising result—and to determine whether any different ratio between skin and rectal temperatures would better describe storage phenomena—we conducted two special experiments extending over longer periods of time and with more frequent successive observations throughout the period involved.

In both these experiments, the air temperature was maintained as closely as possible at the skin temperature of the subjects, so that convection loss ranged only from −3 to +4 kilogram calories per square meter per hour. The actual air temperature varied from 31.9° to 35.3°C. In experiment A, the wall temperatures started at 37.1°C. and were kept between that point and 36.9°C. for 100 minutes. The wall temperature was then rapidly reduced to 25.8°C. at 140 minutes, 19.9° at 160 minutes, and 18.2° at 180 minutes. It was then maintained between 17.1° and 17.8° until the close of the experiment at 360 minutes. Thus, the operative temperature averaged 36.1° during the first 120 minutes and 24.6° during the last 200 minutes.

In experiment B, the walls were cold throughout, varying between 14.3° and 15.5°C., a condition maintained for 360 minutes. The mean operative temperature in this experiment was 22.9°C.

Figure 3 shows the general results obtained in these two experiments. Each point on the curve represents a 20-minute interval. The curves have been plotted arbitrarily as overlapping, although the two experiments were conducted on different days; and the point at which the walls were cooled in experiment A is indicated by a vertical line.

It will be noted that a slight radiation gain occurred before this point in experiment A, this gain plus the metabolism being balanced by high evaporative heat loss. After this point in experiment A, and throughout experiment B, there was a large loss of heat by radiation to cold walls. Convection interchange was negligible throughout. Metabolism fell at the beginning of each experiment (as is usual) and, later, rose slightly in experiment A and much more definitely in experiment B.

During the hot phase of experiment A, skin temperature fell very slightly and rectal temperature rose. During the cold phase of this experiment, both skin and rectal temperature dropped, but both became stabilized during the last hour. During experiment B, both skin tempera-

ture and rectal temperature dropped; but again, both were essentially stabilized during the final hour.

In figure 4 we have presented regional temperatures for the fifteen points on the body surface studied. It will be noted that for the last hour of experiment A every region showed a stable or rising skin temperature

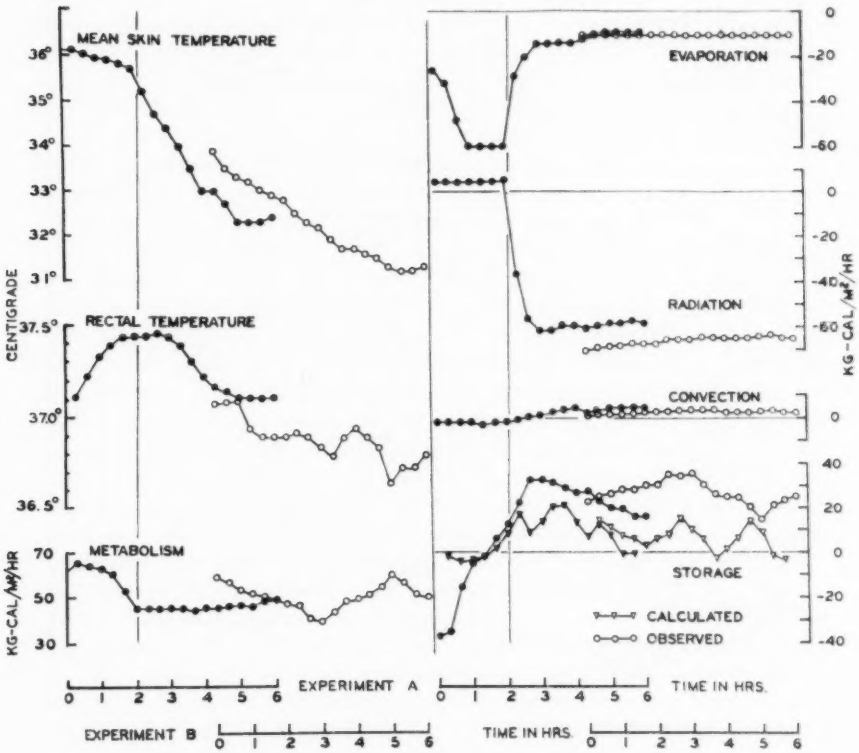

Fig. 3. Progressive temperature changes and changes in the factors of thermal balance on exposure to a cold environment.

Experiment A. Operative temperature of 36°C. for the first 2 hours, operative temperature of 25°C. for the last 4 hours.

Experiment B. Operative temperature of 23°C. for the entire 6 hours.

(for the last three 20-min. periods) except the chest, the back of the calf, and the seat. In experiment B, local skin temperatures were stable or rising for over two hours in the case of the forehead, scalp, fore-arm, shoulder, side-calf, back-calf, and seat; for an hour or more in the case of the cheek, upper arm, hand, chest, trunk, kidney, and thigh; only the instep failed to stabilize its temperature.

During the whole last two hours of this experiment, the rectal temperature fell only from 36.9° to 36.8°; and the mean skin temperature fell only from 31.7° to 31.3°C. Yet during this period the body was producing

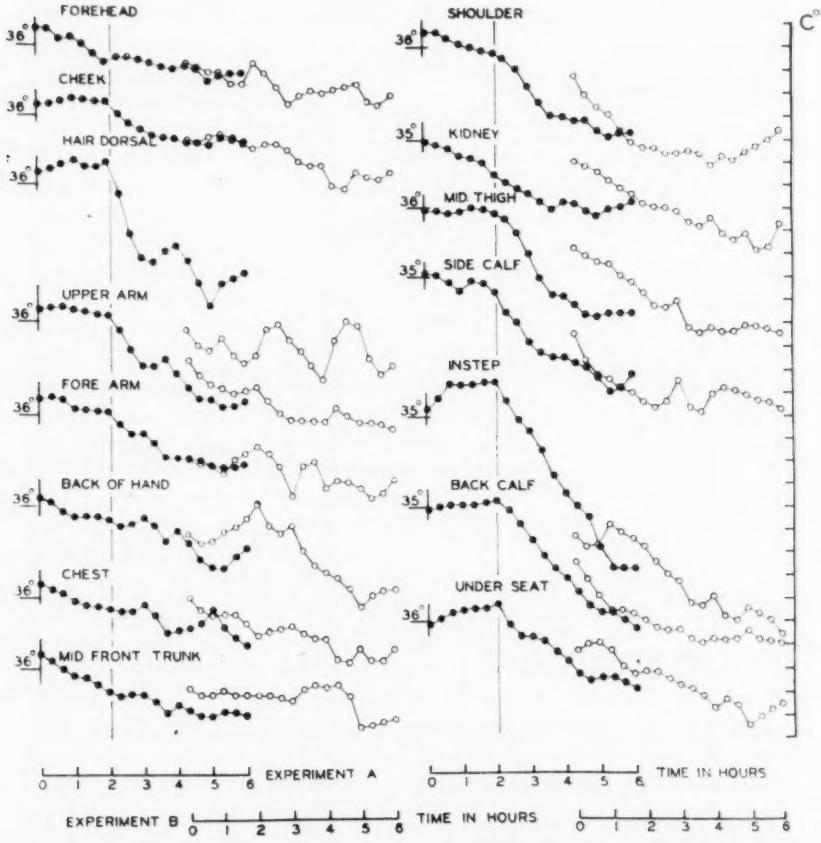

Fig. 4. Progressive changes in temperatures of fifteen regions on the body surface on exposure to a cold environment.

Experiment A. Operative temperature of 36°C. for the first 2 hours, operative temperature of 25°C. for the last 4 hours.

Experiment B. Operative temperature of 23°C. for the entire 6 hours.

53 kilogram-calories of heat per square meter of body surface per hour by metabolism, gaining 2 kilogram-calories by convection, losing 11 kilogram-calories by evaporation, and 65 by radiation—leaving 21 kilogram-calories which must have been stored by cooling of body tissues. The graph at

the bottom of figure 3 shows the storage throughout the experiments as computed in this way by difference and (triangles) the storage as computed from changes in skin temperature and rectal temperature weighted respectively in the ratio of 1:2.

It seems clear from this graph that the 1:2 ratio gives no adequate picture of storage. Furthermore, from the data cited above, as to stable skin and rectal temperatures maintained for considerable periods while the body was actually cooling off rapidly, it seems clear that no reasonable weighting system can be used which will represent what happens when the body is subjected to severe chilling. With moderate atmospheric conditions the 1:2 ratio may be useful (Winslow, Herrington and Gagge, 1939). Under more extreme conditions, however (both on the hot and the cold side), we are forced to conclude that the vaso-motor system has an extraordinary power of stabilizing both rectal and skin temperature while considerable chilling of less vascular tissues is going on. This cannot, obviously, proceed for an indefinite period; and toward the end of experiment B we have evidence that a compensatory increase in metabolism has begun. It may well be that the stimulus to this increased metabolism arises from the chilling of the muscular tissues themselves; independent of the relatively stable temperatures of the blood stream.

It may be of interest to note that, during the hot phase of experiment A, the difference between rectal and skin temperature rose from 1° to 1.7°C. and the conductance rose from 24 to 40 and fell again to 33. During the cold phase, the $T_R - T_S$ differential rose to 4.8° and the conductance value fell to 13—both these values remaining constant for the final hour. In experiment B, the $T_R - T_S$ differential rose from 3.2° to 5.5°C. and the conductance fell from 26 to between 13 and 14. Again, these values were constant for the final hour.

Specific influence of walls and air. After this essential discussion of basic fundamentals, we may now turn to the major objective of the present study, the differential influence of wall temperatures above and below air temperatures.

Our basic data are presented in table 1, each horizontal line representing the average of two experiments on each of the two subjects studied. Four sets of experiments were made with walls about 12°C. warmer than the air, eleven sets with air and walls equal, six sets with walls 10° to 12° cooler than the air, and five sets with walls 16° to 20° colder than the air.

The results are presented graphically in figure 5 for the average data for the period from 40 to 200 minutes.

The data for radiation and convection heat interchanges (in the upper right of the chart) show how successfully these factors were varied over a range of some 50 kilogram-calories per square meter of body surface for the same operative temperature. The graphs for metabolism and evapo-

ration show essentially identical results for all four experimental conditions.

Skin and rectal temperatures are the same at a given operative temperature for the equal and cool and cold wall conditions. Both these tempera-

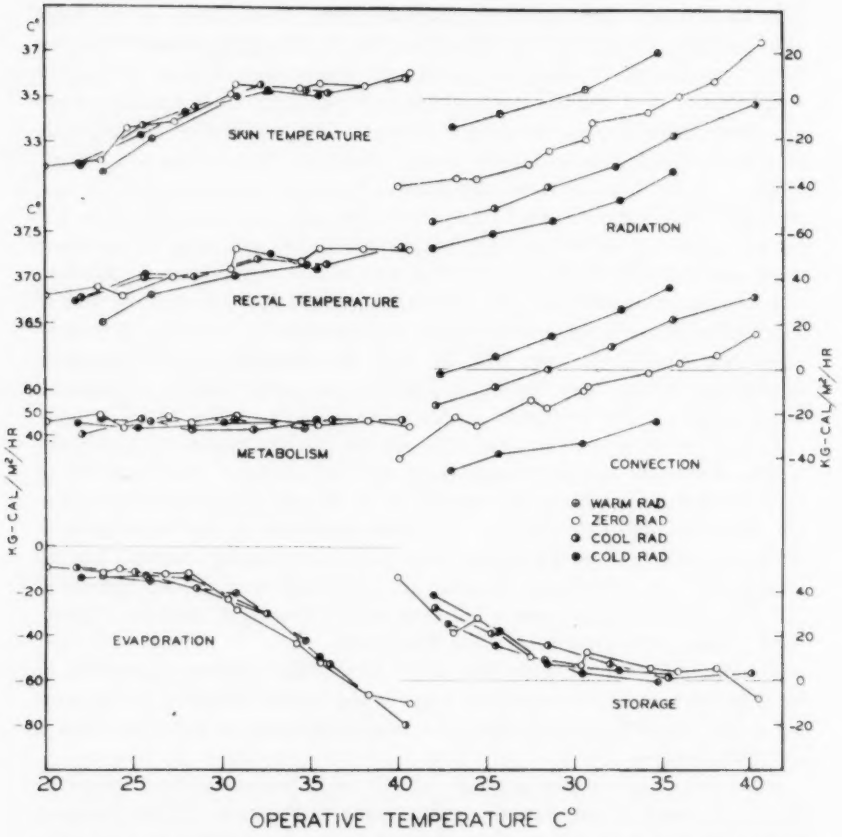

Fig. 5. Temperature values and values for various factors in thermal balance plotted against operative temperature with mean radiant temperatures above, equal to, and below air temperatures.

tures are slightly lower, at a given operative temperature, with the cold-air warm-wall situation and storage for this experimental condition is very slightly reduced as a result of this lower skin temperature.

These results, then, confirm in a qualitative sense those reported in an earlier communication (Herrington, Winslow and Gagge, 1937). The re-

duction in skin temperature associated with the cold-air warm-wall situation is, however, much less (0.5°–1.0°) than in our earlier studies (1°–2°). In the previous study, a constant rate of air movement was assumed. In the present studies air movement was recorded for each experiment; and it may be seen from table 1 that at an operative temperature of 40° air velocity was between 6 and 7 cm. per second, while at 30° the corresponding value was about 8 cm., and at 20° nearly 11 cm. If this occurred in our new booth, the phenomenon must have been much more marked in the smaller, old booth used in 1937. We are inclined, therefore, to believe that the earlier results were influenced by increased air movement at low operative temperatures. We may conclude from our present studies that the cold-air warm-wall situation does exert a cooling influence greater than would be predicted from its operative temperature, but that the difference is slight. It is evident that the influence exerted has nothing to do with specific effects of radiation and convection since a hot-air cold-wall situation (even with a 20° differential) gives results identical with those of equal wall and air. The phenomenon must be due to an influence of cold air alone. The magnitude of the effect is such that it can easily be explained, as we have suggested, in a study on clothed subjects (Winslow, Herrington and Gagge, 1938) "by local chilling of the membranes of the upper respiratory tract (which might be far more effective in stimulating vaso-constriction than would be expected from its direct effect on calorie loss); by local chilling of the body by the metal supports of the chair in which the subject reclines (although the seat of the chair is cloth); and by temporary increases in convection loss when the subject enters the room or moves his arms or body."

CONCLUSIONS

1. A new experimental booth is described, which provides more accurate control of the various experimental variables in heat interchange than was previously possible.

2. Radiation areas as determined in this new booth are found to lie between 70 and 75 per cent of the DuBois area.

3. Convection constants are found to lie close to a value of 1.0 multiplied by the square root of air velocity in centimeters per second.

4. It is shown that, when the body is subjected to markedly cold or hot conditions, storage can not be estimated from changes in skin temperature and rectal temperature. Both these temperatures may be maintained by the vaso-motor system at a stable level for considerable periods, in spite of the fact that appreciable heating or chilling of less vascular tissues is taking place. Storage, under such conditions, can be measured most adequately by difference—from the algebraic sum of metabolic heat production, evaporative heat loss and gain, or loss by radiation and convection.

5. In general, the influence of air and walls is accurately represented by the fundamental concept of operative temperature as previously advanced by us. So far as cold-wall hot-air situations and equal wall and air situations are concerned, physiological responses exactly follow operative temperature (as computed from mean radiant temperature and radiation area combined with air temperature, air movement, and convection constant). With a cold-air hot-wall situation, however, skin and rectal temperatures are somewhat lower—for a given operative temperature—than with equal wall and air or with hot-air and cold walls. The difference is slight (0.5°–1.0°) and can be accounted for by the cooling effect of the cold air upon the membranes of the nose and throat, and, perhaps, also by additional heat loss to the metal supports of the subject's chair and to increased convective heat loss due to movements of the arms and legs.

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STANDARD OPERATIVE TEMPERATURE, A GENERALIZED TEMPERATURE SCALE, APPLICABLE TO DIRECT AND PARTITIONAL CALORIMETRY

A. P. GAGGE

Contribution no. 30, from the John B. Pierce Laboratory of Hygiene, New Haven, Conn.

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In view of the trend[1] in recent years among workers in the general field of metabolism and calorimetry to emphasize the physiology of heat elimination from the animal body, and since such processes of heat elimination are so fundamentally governed by the physical properties of the environment itself, a generalized simplification and standardization of these properties seems in order. It is the purpose of the present paper to define and derive a generalized environmental temperature scale, that combines as a single measurement certain of the thermal effects of the physical environment, aqueous or atmospheric, and in the latter case for any combination of radiant temperature, ambient air temperature and air movement. Use of such a generalized temperature scale as an independent variable would give to experimenters working under diverse conditions a common reference measurement—and one which permits differentiation between effects of a purely physical nature and those of a physiological nature.

DEFINITION AND DERIVATION. The formal basis of standard operative temperature, T_o, is Newton's Law of Cooling and this factor is defined as the equivalent environmental temperature with which a warm body (such as a human or animal subject) with surface temperature, T_s, exchanges heat at a *standard* cooling rate, K_o. From Newton's Law

$$\text{Heat Loss to Environment} = K_o\,(T_s - T_o), \tag{1}$$

our definition may be written

$$T_o = T_s - (\text{Heat Loss to Environment})/K_o. \tag{2}$$

The above definition (2) is universally applicable to all types of Calorimetry, where the Calorie loss (aside from evaporative cooling) from the animal subject is measured without particular attention to its mode of elimination. By environment one means the physical complex actually

[1] Recent comprehensive reviews in the literature indicating this trend are given by Deighton (1933), Kleiber (1937), Murlin (1938), Burton (1939) and Dill (1939).

surrounding the animal; it may be anything from a water bath to a respiration chamber.

In Partitional Calorimetry (Winslow, Herrington and Gagge, 1936), where the mode of heat loss to the environment is of primary interest, the factors, mean radiant wall temperature (T_W), ambient air temperature (T_A) and air movement, V, must be considered. Relative humidity is omitted from the present discussion as it affects only the efficiency of evaporative cooling, a regulative process separately measurable and distinct from the direct heat loss to the environment by radiation and convection.

For the present let us consider an environment where air movement is constant; under this condition loss by radiation and convection for the animal subject may be expressed as

$$R + C = (A_R/A_D)\, k_r\, (T_S^4 - T_W^4) + K_C(T_S - T_A), \tag{3}$$

where temperatures are absolute in the 4th power radiation Law, k_r and K_C are the universal radiation constant (4.92×10^{-8} kg.cal./m.^{2}/hr./°C.) and total convection constant respectively, and A_R/A_D is the ratio of the radiation area of the animal subject to its total area. As a first approximation (3) may be rewritten

$$R + C = K_R\, (T_S - T_W) + K_C\, (T_S - T_A), \tag{4}$$

which equation on substitution in our definition (1) or (2) gives

$$T_O = \frac{K_R T_W + K_C T_A}{K_R + K_C}, \tag{5}$$

and

$$K_O = K_R + K_C. \tag{6}$$

From (5) Operative Temperature is actually the average of wall and air temperatures weighted according to their relative effectiveness as described by the radiation and convection constants. This special application of operative temperature was first used in Partitional Calorimetry (Winslow, Herrington and Gagge, 1937a; Herrington, Winslow and Gagge, 1937) to combine as a single variable the temperature equivalents of the radiative and convective environments.

It has now been shown experimentally (Winslow, Gagge and Herrington, 1939) that the loss by convection is proportional to the square root of the air movement for the human body in a semi-reclining position up to air movements of 250 cm. per second. It is reasonable to assume that this relationship is generally true for any body size or position within the above limits of air movement, a limit considerably higher than normal under indoor conditions. Let us ask at what temperature, T_A', at standard air

movement, V_o, does one lose the same heat by convection as at the observed temperature, T_A, and air movement, V. From the equation for convection loss

$$k_c\sqrt{V}(T_S - T_A) = k_c\sqrt{V_o}(T_S - T_A'),$$

we have

$$T_A' = \sqrt{V/V_o}\,T_A - (\sqrt{V/V_o} - 1)\,T_S. \tag{7}$$

The presence of T_S in (7) is necessary since T_A' is equal to T_A unless the surface temperature of the subject is different from the air temperature itself.

If we now assume that (5) is valid for a standard air movement, V_o, by substituting (7) in (5) we have the operative temperature in terms of T_W, T_A, and V.

$$T_o = \frac{K_R}{K_o}[T_W] + \frac{K_C}{K_o}[(\sqrt{V/V_o})\,T_A - (\sqrt{V/V_o} - 1)\,T_S], \tag{8}$$

where the standard cooling rate, K_o, is equal to

$$K_R + k_c\sqrt{V_o}. \tag{9}$$

(8) describes the equivalent temperature in which a subject, with the observed surface temperature, T_S, would lose the same amount of heat at a standard cooling rate, K_o, as by radiation and convection in the original environment.

By standardizing K_o and its associated air movement, V_o, a temperature scale is established on a sound calorimetric basis, if one measures first the surface temperature of the animal subject and, secondly, either the total heat loss to the environment by direct calorimetry or the individual factors, wall temperature, air temperature, and air movement, themselves.

Application. Application of the methods described above will be limited for the sake of brevity to two experimental cases with the human body; 1, comparison of certain data taken in a Water Bath Calorimeter (Burton and Bazett, 1936) with those of the Russell Sage Calorimeter (Hardy and DuBois, 1937), and 2, comparison of unpublished data from this laboratory taken in the Partitional Calorimeter for various degrees of air movement when air and wall temperature are equal. The third possibility, the condition in which wall and air temperature independently vary has been already described in the literature (Herrington, Winslow and Gagge, 1937; Winslow, Herrington and Gagge, 1938; and Winslow, Gagge and Herrington, 1940).

Case 1. In both the Water Bath Calorimeter and the Russell Sage Calorimeter an evaluation of skin temperature is possible. In the former, since the bath is extremely well stirred, the bath temperature is practically

equal to the skin temperature, while in the latter a direct measurement is possible. For both, the total calorie loss by the body to the environment is measured directly. In each case the requirements necessary to derive standard operative temperature as originally defined by (2) are satisfied.

In table 1 are presented data derived from those obtained for a subject in the Water Bath Calorimeter by Burton and Bazett. The value of operative temperature has been calculated from equation (2), using a factor 5.3 kgm.cal./m.²/hr./°C., for K_o, a value equal to the cooling constant

TABLE 1

*Calculation of operative temperature for data on subject H C B in water bath calorimeter**

CONDUCTANCE	HEAT LOSS TO BATH	BATH TEMP.	HEAT LOSS ÷ 5.3	T_O	CONDUCTANCE	HEAT LOSS TO BATH	BATH TEMP.	HEAT LOSS ÷ 5.3	T_O
kg. cal. / sq. m./hr./ °C.	*kg. cal. / sq. m./hr./ °C.*	*°C.*	*°C.*	*°C.*	*kg. cal. / sq. m/hr./ °C.*	*kg. cal. / sq. m./hr./ °C.*	*°C.*	*°C.*	*°C.*
17.7	27.6	35.35	5.21	30.14	29.3	25.8	35.52	4.87	30.65
9.8	44.7	32.05	8.43	23.62	28.2	28.5	35.52	5.38	30.14
34.0	38.8	35.88	7.32	28.56	28.8	29.1	35.52	5.49	30.03
28.5	33.0	35.88	6.23	29.65	7.6	33.5	32.26	6.32	25.94
11.9	66.3	31.26	12.51	18.75	7.6	32.0	32.26	6.04	26.22
10.6	57.1	31.26	10.77	20.49	7.9	31.8	32.26	6.00	26.26
36.6	25.2	35.92	4.76	31.16	22.5	19.6	36.26	3.70	32.56
9.5	32.1	33.12	6.06	27.06	21.4	22.7	36.26	4.28	31.98
8.7	28.1	33.12	5.30	27.82	15.9	38.0	34.34	7.17	27.17
7.9	24.8	33.12	4.68	28.44	15.2	34.0	34.34	6.42	27.92
10.9	30.6	33.72	5.77	27.95	10.9	24.1	34.34	4.55	29.79
10.9	30.1	33.72	5.68	28.04	10.9	32.0	33.62	6.04	27.58
11.9	32.5	33.72	6.13	27.59	10.9	31.2	33.62	5.89	27.73
10.1	27.1	33.72	5.11	28.61	10.1	35.1	33.01	6.62	26.39
17.0	107.2	30.22	20.20	10.02	6.7	22.5	33.00	4.25	28.75
11.9	71.6	30.27	13.51	16.76	10.6	28.6	33.57	5.40	28.17
11.9	69.7	30.28	13.15	17.13	11.3	30.6	33.57	5.77	27.80
11.9	68.5	30.28	12.93	17.35	22.6	29.2	35.03	5.51	29.52
11.7	66.5	30.29	12.55	17.74	21.2	28.8	35.03	5.43	29.60

* Note: the author is indebted to Doctor Burton and Doctor Bazett for the use of the original data from their publication (1936) in preparation of the above table.

of the Russell Sage Calorimeter. In figure 1 the physiological variable, conductance (Burton and Bazett, 1936; Winslow, Herrington and Gagge, 1937a), a measure roughly proportional to the peripheral circulation of the skin, is plotted against operative temperature for a subject in each calorimeter. The data for the Russell Sage subject are taken directly from the literature (Hardy and DuBois, 1937) to which the reader is referred. Thus, figure 1 compares the vascular response of two subjects in widely different types of calorimeters on a common operative temperature scale (in this particular case the temperature of the Russell Sage

Calorimeter is also the operative temperature). The following conclusions may be reached from the figure.

a. Vascular regulation or, rather, vasodilatation begins to increase approximately at the same operative temperature in spite of the fact that

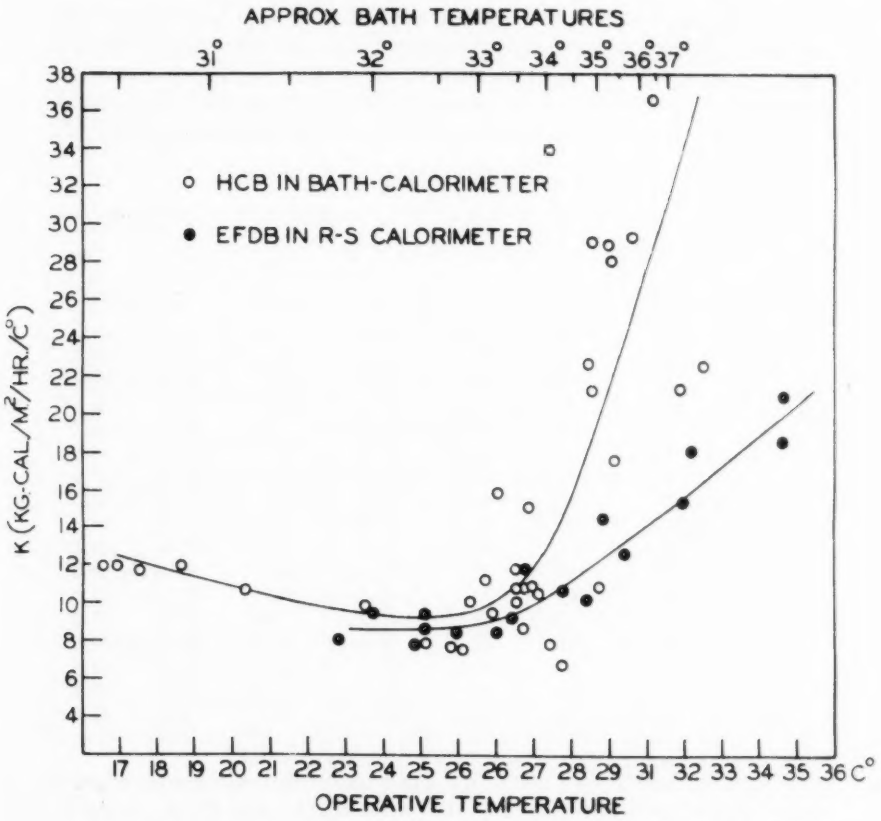

Fig. 1. The relation between conductance (a measure of peripheral blood flow) and operative temperature for a subject (EFDB) in the DuBois-Hardy Calorimeter, and another (HCB) in the Burton-Bazett Water Bath Calorimeter.

the media of heat loss are quite different. This fact suggests that vascular regulation in the two instances results from the same thermal stimulus.

b. The large difference in the two cases between the rate of rise in peripheral circulation with increasing operative temperature indicates the relative economy in vascular effort provided by the process of evaporative

regulation which, while present in the Russell Sage Calorimeter, is absent in a water bath.

Case 2. A series of data averaged from those taken on two male subjects in a semi-reclining position under conditions of equal wall and air but under various air movements is presented in table 2. In the first three columns are presented the basic physiological data, metabolism (M), evaporation loss (E), and skin temperature (T_S). In the next two columns the air temperature (equal to that of the wall) and air movement are recorded. In the sixth column the equivalent air temperature for a standard air movement of 7.6 cm. per second is calculated from equation (7).

TABLE 2

Calculation of operative temperature (V_O = 7.6) for data where air movement varies

CONDUCTANCE	M	E	T_S	$T_A = T_W$	V	T'_A	T_O
kg.-cal. / sq. m.-hr.-°C.	*kg.-cal. / sq. m.-hr.*	*kg.-cal. / sq. m.-hr.*	*°C.*	*°C.*	*cm./sec.*	*°C.*	*°C.*
1	52	49	35.9	35.3	7.6	35.3	35.3
2	46	44	35.1	35.1	51	35.1	35.1
3	48	42	35.1	35.3	132	35.9	35.6
4	49	44	35.2	35.3	264	35.8	35.6
5	47	30	35.5	33.1	7.6	33.1	33.1
6	48	21	35.5	33.4	51	30.1	31.8
7	50	16	35.3	33.7	132	28.6	31.2
8	45	16	35.2	33.9	264	27.5	30.7
9	46	21	34.9	31.3	7.6	31.3	31.3
10	46	16	33.8	31.4	51	27.6	29.5
11	47	13	33.0	31.4	132	26.3	28.9
12	50	14	33.1	31.4	264	23.1	27.3
13	43	13	34.1	27.3	7.6	27.3	27.3
14	50	12	32.1	27.6	51	20.4	24.0
15	49	12	31.3	27.7	132	16.3	22.0
17	52	12	32.6	22.4	7.6	22.4	22.4
18	54	12	31.2	22.3	51	8.1	12.5

The data in table 2 were taken in a small partitional calorimeter where a value of 0.5 is found for each of the ratios K_R/K_O and K_C/K_O, when V_o equal 7.6 cm. per second. In the final column of the table the standard operative temperature is calculated from equation (6). The basic physiological data are plotted against operative temperature in figure 2, from which the following conclusions may be reached.

a. Metabolism is approximately constant within the temperature range studied.

b. After the data have been "normalized" by operative temperature, evaporative regulation begins at 31°C., this fact being true for all degrees of air movement. As evaporative regulation is a very sensitive index of any

change in heat balance with the environment, it is specially significant that this measure is completely "normalized" by operative temperature.

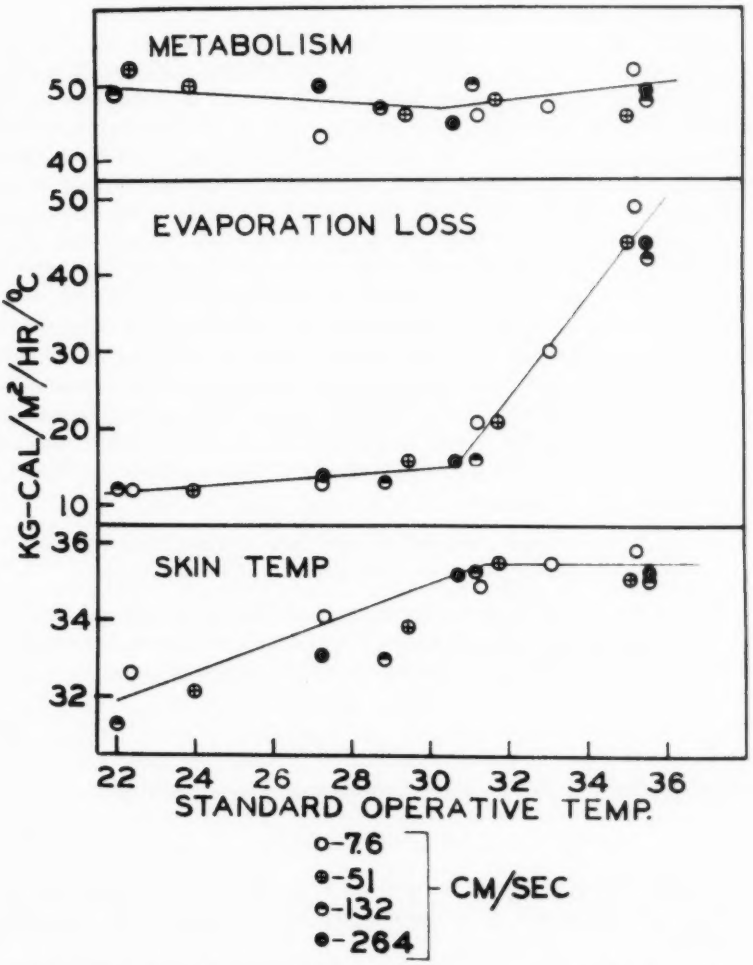

Fig. 2. The relation between metabolism, evaporation loss and skin temperature and operative temperature for various air movements and dry bulb temperatures in the Partitional Calorimeter.

c. The skin temperature curves also inflect at the critical point of 31°C. operative temperature, further indicating normalization.

d. The fact that, at skin temperatures below 31°C., points for high air movement but for the same operative temperature lie below the graph may indicate a vascular response to air movement influenced by other than thermal factors.

Standard K_o. In the original presentation of the two experimental cases discussed in the previous paragraphs no effort was made to generalize the use of operative temperature. In the first case, two different experimental methods of calorimetry were compared on a temperature scale derived from one of them. In the second case, a family of experiments similar in method but under different conditions, were reduced to a common independent variable. The real value of the concept of operative temperature should lie in its ability to reduce all methods of calorimetry to a common single independent variable. This is apparently possible by defining and standardizing on a value of the cooling constant, K_o.

Let us define the standard value for K_o for a given animal subject to be the value of its Newton Cooling constant when it is placed under basal conditions in an ideal environment, where the wall and air temperatures are equal, where air movement is unity, where heat production exactly balances heat loss to the environment, and where neither evaporative nor vascular regulation are necessary. For the human body in thermal equilibrium

$$K_o = \frac{\text{Basal metabolism less basal evaporation}}{\text{Difference between skin and air-wall temperature}}.$$

According to our definition, if we assume a value of 40 kgm.cal./M.²/hr. for the basal metabolism, 10 kgm.cal./M²/hr. for the basal evaporation, and 35°C. for the optimum value for the skin temperature, we have

$$K_o = \frac{30}{35^\circ - T_A}. \tag{10}$$

From equation (9) above it follows for unit air movement that

$$K_o = K_R + k_c. \tag{11}$$

K_R, strictly speaking, is not a constant since the true radiation law is a fourth power difference, not a linear difference. A more exact expression for (11) is

$$K_o = \left[4k \times (A_R/A_D) \times \frac{T_W^3 + T_S^3}{2}\right] + [k_c], \tag{12}$$

whose terms have already been defined above.

Recent experiments (Winslow, Gagge and Herrington, 1940) have shown that the most probable value for A_R/A_D is 75 per cent and the value for

k_c is 1.0 when V is expressed in centimeters per second. In the light of our earlier substitutions (12) now becomes

$$K_O = 0.75 \times (4 \times 4.92 \times 10^{-8}) \times \left[\frac{T_W^3 + (273° + 35°)^3}{2}\right] + 1.0. \quad (13)$$

Both (10) and (13) are numerically satisfied simultaneously when K_O and T_W are equal to 5.2 and 29.2°, respectively. Therefore, in an ideal environment of 29.2°C. with unit air movement, the value of the standard cooling constant, K_O, is found to be 5.2 kgm.cal./m.²/hr. Under these same ideal conditions, K_R equals 4.2, and K_C equals 1.0.

The value for standard K_O of 5.2 kgm.cal./m.²/hr./°C. is extremely close to the cooling constant of 5.3 of the Russell Sage Calorimeter. This near equality, although fortuitous, is also fortunate as it gives added significance to the former value. Instead of referring operative temperature to a cooling rate under an ideal condition, one could give it more realistic meaning by referring to a cooling rate and a temperature, equivalent to that in a calorimeter of known and established physical properties.

In conclusion, a biophysical temperature scale for the human body generally applicable to all forms of calorimetry may be defined by either of the two relations

$$T_O = T_S - (\text{Heat Loss to the Environment})/5.2, \quad (14)$$

$$T_O = 0.81\,[T_W] + 0.19\,[\sqrt{V}\,T_A - (\sqrt{V} - 1)\,T_S], \quad (15)$$

where heat loss is expressed in kilogram-calories per square meter per hour and when V is measured in centimeters per second.

Discussion. The one physical property of the environment not included in operative temperature is relative humidity. Except under, perhaps, extremely warm saturated conditions in which no animal could long exist, the effect of humidity is of secondary importance on absorption of radiation and conductivity of the surrounding air and hence on the radiation and convection constants themselves. It is not believed necessary to include this effect in operative temperature. Humidity, however, does affect insensible perspiration and evaporative regulation. Its direct effect is to change the rates of secretion and evaporation, any change in which can only result in a change in mean skin temperature. However, as mean skin temperature is fundamental to our definition of operative temperature, operative temperature must include in its interpretation these secondary effects of humidity on the heat exchange by radiation and convection with the environment.

From partitional calorimetry studies (Gagge, Winslow and Herrington, 1938) on the human body it has been shown that, when evaporative regulation is completely successful, this loss increases by exactly the same amount as the calorie demand (loss by radiation and convection) of the environ-

ment decreases, and vice versa. The statement is also true for variations in humidity (Winslow, Herrington and Gagge, 1937b), as long as the evaporative rate is under 30 kilogram-calories per square meter per hour per centimeter of the Hg vapor pressure difference between skin and air—a value found to be the maximum effective cooling rate of the human body (Gagge, 1937). Because of this very exact correlation between evaporative regulation and calorie demand, and since calorie demand (or heat loss to the environment) is also fundamental to our definition of operative temperature, it may be generally stated that evaporation within the range of complete regulation is variant only with operative temperature and invariant with any of the individual physical factors of which it is composed.

The experimental validation of this latter conclusion for a subject at rest is found for T_o environments where wall and air temperature are independently varied (Winslow, Herrington and Gagge, 1937b), where air movement varies (see fig. 2 of this paper), and finally where humidity varies (Winslow, Herrington and Gagge, 1937a).

Operative temperature actually is a formal analytic expression, defined and derived from a well known physical principle. It is, strictly speaking, a *calorimetric* temperature. Until actually verified by experiment it implies no exact relation to the sensation of temperature or to the sensory equivalance in terms of hot or cold of the physical components of the environment. As such it is only a step to an end. The "Effective Temperature Scale" (Houghten and Yagloglou, 1923, 1924; Yaglou and Miller, 1925), sponsored by the American Society of Heating and Ventilating Engineers and currently used by many physiologists, is derived experimentally on a straight sensory basis. It combines in a single variable the sensory effect of not only air movement but also humidity. "Operative" and "Effective" temperatures must obviously have many properties and trends in common but the difference between the two scales is fundamental—the former is based on a known physical law, while the latter is derived from an experimental procedure where the sensations of temperature and comfort are the only discriminating factors defining the scale. From the latter one can make no accurate deduction in a calorimetric or thermodynamic sense nor are general physiological responses to it any more certain than to operative temperature. Somewhere between "Operative Temperature," analytically a pure calorimetric scale, and "Effective Temperature," experimentally a pure sensory scale, must lie a temperature scale to which all physiological responses, vascular, sensory, and metabolic are related—a truly physiological temperature scale.

From the preceding section it is quite apparent that operative temperature is essentially a biophysical quantity since skin temperature is included in its definition. It is a measure of the thermometric potential that governs the calorie exchange between the body and its surrounding en-

vironment, and at the same time it reduces this heat exchange to an equivalent temperature drop along a standard gradient from the body surface. By equilibration and standardization of the most complex environments all calorimetric procedures may be described in terms of a common reference scale, and with this scale it is possible to separate from any physiological temperature response its true physical component.

SUMMARY

Standard operative temperature based on Newton's Law of Cooling is defined as the equivalent environmental temperature with which a warm body (such as a human or animal subject) with surface temperature, T_s, exchanges heat at a *standard* cooling rate (excluding evaporation effects). Equations are derived from which one may calculate operative temperature when the skin temperature is measured and either the heat loss to the environment or the environmental factors wall temperature, air temperature, and air movement, are known. Operative temperature is a measure of the thermometric potential that governs the calorie exchange between the body and its surrounding environment. It also includes any humidity effect of the environment on this exchange. It is shown that the loss by evaporative regulation is variant only with operative temperature and invariant with any of the individual physical factors of which it is composed. Use of operative temperature is suggested as a standard reference scale for direct and partitional calorimetry and when one wishes to differentiate between effects of a purely physical nature and those of a physiological nature.

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FACTORS DETERMINING THE PRODUCTION OF VENTRICULAR FIBRILLATION BY DIRECT CURRENTS (WITH A NOTE ON CHRONAXIE)[1]

RENÉ WÉGRIA[2] AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

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In 1938, one of us (1) suggested that the sensitivity of the ventricle to fibrillating agents might perhaps be established quantatively by determining the strength of a brief shock which causes fibrillation when applied late in systole. Before such a criterion could be adopted, it was necessary to prove that such a localized brief stimulus causes fibrillation only when applied during this phase of the heart cycle; in other words, that the late portion of systole (ca 0.06 sec.) is a peculiarly vulnerable period. In a previous communication (2) we presented evidence that this is true for condenser as well as induction shocks. However, we soon found that the fibrillating action of somewhat longer D.C. and A.C. shocks is not limited to this period; indeed, under certain conditions, stimuli cause fibrillation when their effective portions fall entirely during diastole. It therefore became important to investigate the mechanisms of action of such D.C. and A.C. shocks in order to determine whether the fibrillating threshold to brief shocks is a valid general test for the sensitivity of the ventricle to fibrillating agents.

The present communication deals with the conditions under which fibrillation is produced *a*, by short rectilinear shocks which, temporally speaking, can fit into this period, and *b*, by direct currents of longer duration, which lap over the vulnerable phase. As regards the latter, experimental study has shown that our *a priori* conclusions regarding the stimulating action of such currents prove correct. However, experiment has brought into evidence additional factors which could not have been predicted by theoretical reasoning.

PROCEDURE. *Apparatus.* Numerous observations were made on 16 dogs. They were anesthetized, usually with morphine and sodium barbital; the chest was opened in the midline and the slit pericardium was

[1] This investigation was supported by a grant from the John and Mary R. Markle Foundation.

[2] Fellow of the C. R. B. Educational Foundation.

stitched to the retracted chest opening. Thus, the heart was suspended in a pericardial cradle so that its position was influenced little by artificial inflation of the lungs. It may be noted parenthetically that, while such suspension of the heart is useful in precise stimulation of ventricular surfaces, the abnormal contacts between the heart, lungs and chest wall may modify the form of electrocardiograms recorded by standard leads. This, rather than abnormalities in the heart itself, accounts for some modifications in the configuration of our electrographic curves. However, these curves were always sufficiently distinctive to differentiate between normal and aberrant types of conduction.

Direct currents of various strengths were applied for intervals of 0.01 to 0.33 second to a definite anatomical spot of the ventricular surface. Such stimuli were given every sixth beat in order to allow full recovery in rhythm and dynamics of cardiac action. The closing and opening of the circuit were arranged so as to advance or recede slightly with respect to the cardiac cycle in successive stimulations. In the majority of experiments, bipolar, ball-tipped, Ag-AgCl electrodes about 8 mm. apart and prepared after the method of Langelaan (3) were used. Such solid electrodes have the advantages 1, that they can be pressed firmly against the ventricular surface without causing mechanical stimulation, with avoidance of a variable "apparent" resistance with movements of the heart, and 2, that they offer less resistance to direct current than fluid electrodes, thereby reducing the voltages necessary for fibrillation and diminishing the tendency for currents to spread.

Unfortunately, with potentials of 10 volts or over, and particularly when durations of current equal 0.10 second or more, slight polarization does occur. Cumulative effects of repeated stimulations were avoided by meticulously changing the direction of current in successive stimulations by an automatic pole changer.

The duration of the rectilinear shocks and their progressively changing relation to the cardiac cycle were determined by a rotating stimulator (fig. 1, *R*), set to operate ever so slightly out of phase with the heart beat. The speed of the rotator was first set by applying very brief weak shocks (ca, 1 M.A./0.02 sec.) to the ventricle, until a whole series (7–15) caused no effect and another similar series of shocks all evoked premature contractions. When records were taken, it was then found that the former fell during successive moments of systole and the latter during progressive moments of diastole. As a rule, a succession of 9 or 10 effective and the same number of ineffective shocks were sufficient for exploratory coverage of the cardiac cycle. When the tempo of the stimulator had thus been set, the duration of the shock was set by means of the rotating cylinder, *R*, with a longitudinal V-shaped gap which broke the contacts of two smooth tungsten surfaces for variable intervals. Such an initial opening rather than

closing current was employed because it is mechanically much easier to open a circuit than it is to close it evenly for a brief and constant interval.

The difficulty of sparking and uneven contact surfaces, which so easily frustrates mechanical production of pure rectilinear shocks, was overcome by passing very small currents with rather high voltage through these contacts. As illustrated in figure 1, this was done as follows: During the stimulation interval desired, the rotating cylinder, *R*, broke the circuit of a bias voltage (300 volts) across an RCA 885 gas triode. As long as the contacts remained closed, the tube was so biased that it did not conduct. With the break of the bias voltage through the rotating stimulator, instan-

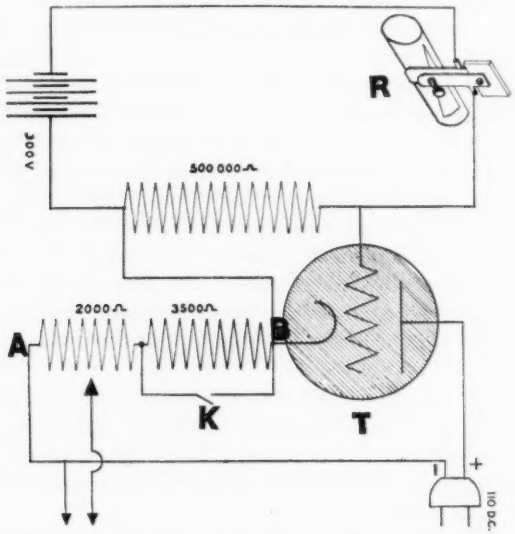


Fig. 1. Schematic diagram of stimulator used. Description in text.

taneous ionization of gas in the triode permitted the tube to conduct a current with a voltage drop of 85 to 90 across the terminals *A-B*. As soon as the bias current was completed again by the rotating stimulator, it ceased to conduct immediately, because the high grid potential (300 V.) was sufficient to cause immediate de-ionization of the gas in the tube. In this way, the duration of conduction by the triode synchronized with that of the contact breaker.

The voltage across *A-B* was reduced to suitable stimulating values by a voltage divider. By closing a key, *K*, voltage ranges from 2 to 80 could be obtained, and by opening this key small variations between 0.1 to 2 volts were realized. The latter proved useful for tests in relating the rhythms of the stimulator and heart.

A left intraventricular pressure curve and a standard electrocardiogram lead were optically recorded by usual methods. A Wiggers type of manometer was used for the former, and a G.E., type A, amplifying electrocardiograph, the galvanometer of which was removed and arranged to write with the pressure curve on 12 cm. film, was found to have decided advantages over string galvanometers in recording the electrocardiograms.

The moment of stimulation was definitely indicated in such electrocardiograms and since temporal relations were chiefly concerned in our studies, this sufficed in our earlier experiments of this series. In later experiments, a G.E., PM-10 oscillograph was introduced into the circuit. This was so arranged that it recorded a 1 mm. deflection per M.A. current, thus giving quantitative data regarding the stimuli used.

Repeated studies of fibrillation—up to 41 in one dog—were made possible by applying as promptly as possible 1 to 7 brief alternating current shocks in succession through padded electrodes. In 225 trials, in this series alone, we had only one failure. It was gradually discovered that the electrodes did not need to be left in place, as reported in a previous paper (2), a matter of importance in recording simultaneous electrocardiograms during observations. As a rule, normal beats were restored after about 1 minute of fibrillation, but we waited an additional 15 to 20 minutes before another test was made.

Results. In order to present our analysis of miles of optical tracings as succinctly and clearly as possible, we are adopting the expedient of referring first to the chart of figure 2 which summarizes our main deductions. We shall follow this with segments of illustrative records, which authenticate our chart, despite the necessity of reducing their size significantly for publication. It may be added that these reproductions of actual records do not represent consecutive beats in any experiment but merely sections of such records, occasionally rearranged slightly in sequence for the sake of logical presentation. This accounts for the apparent failure in reversal of D.C. shocks in the successive segments selected. For the purpose of this presentation, the results are best arranged from the dominant viewpoint of the duration of application rather than intensity of current, it being understood that only currents which are suprathreshold as regards induction of premature contractions or fibrillation are being considered, unless otherwise specified.

I. Short D.C. shocks (ca 0.01 to 0.04 sec.) behave as unitary excitants to the ventricle, i.e., they cause responses which cannot be differentiated from those of brief induction shocks or condenser discharges. In other words, we have found no evidence that *closing* (C) or *opening* (O) of the current have separate stimulating values. This may be due to the fact that the stronger C effect causes a response and the succeeding O effect invariably comes during the refractory period.

As illustrated by line I of figure 2, such shocks applied during the refractory period of systole, *a*, are ineffective regardless of strength.[3] Applied during the vulnerable period, *b*, i.e., during the last 0.03 to 0.06 sec. of mechanical systole and sometimes during the first 0.02 sec. of protodiastole, they elicit an early diastolic premature contraction when they are weak or induce fibrillation when sufficiently strong. The currents which

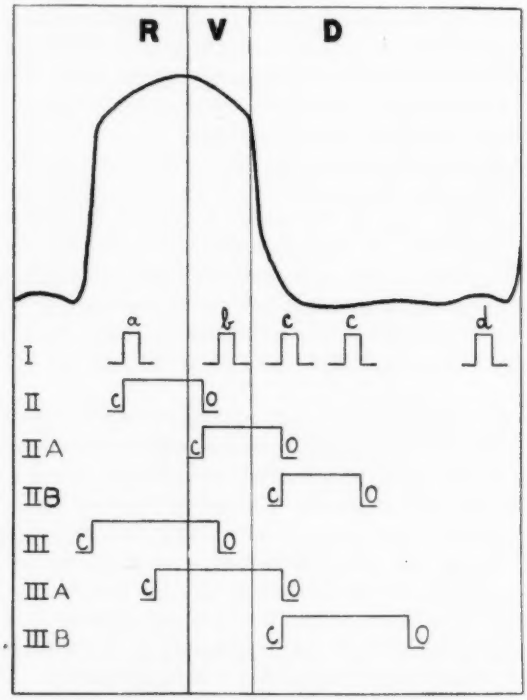


Fig. 2. Diagram indicating the placement of D.C. shocks with reference to a cardiac cycle indicated by a left ventricular pressure curve (upper). *R*—refractory period; *V*, vulnerable period; *D*, diastole. *C*, closing; *O*, opening of D.C. shocks. Further discussion in text.

applied to one locality of the ventricle produce fibrillation, vary slightly in different dogs but remain remarkably constant in repeated trials on the same heart. When such shocks are applied during diastole, *c*, they invariably cause a premature beat, *but never fibrillation*. When such shocks

[3] This statement should be amended to the effect that in 3 experiments out of 90, strong shocks were occasionally effective during this period. In correspondence, King states he has seen a similar undoubted effect.

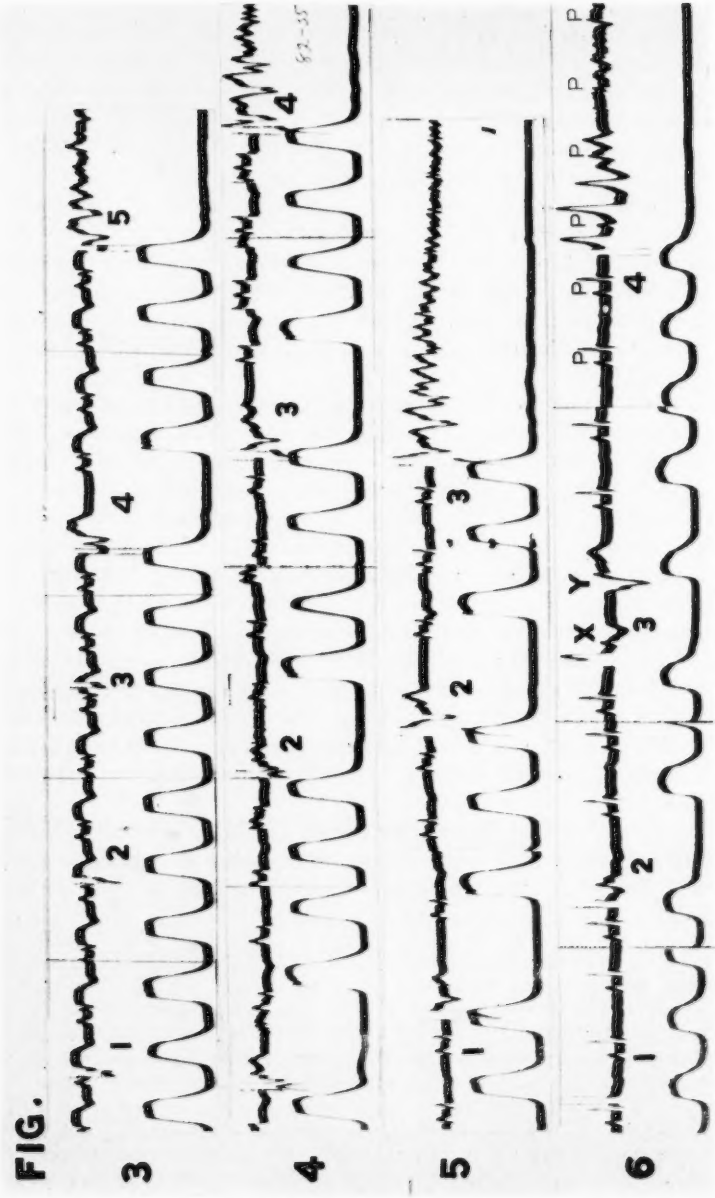
are applied late in diastole, *d*, (i.e., temporally, after the summit of the P wave of normal beats), they are ineffective. This pseudo-refractory phase of late diastole has already been analyzed by one of us (4).

Figure 3 shows segments from an actual record which illustrates some of these reactions induced by shocks 0.04 second in duration. The shocks numbered consecutively show the following reactions: Shock 1 (12 M.A.) applied in diastole causes a large premature beat. Shock 2 (12 M.A.) applied during presystole or pseudo-refractory period of diastole is ineffective, as is shock 3 (12 M.A.) during the refractory period of systole. Shock 4 (12 M.A.) during the vulnerable period gives a premature beat; shock 5 (12 M.A.), a trifle later in the vulnerable period, results in fibrillation. Apparently 12 M.A. represents a critical current for induction of fibrillation. Currents below this were ineffective throughout the experiment.[4]

II. Direct currents of somewhat longer duration (ca. 0.05-0.09 sec.) frequently produce effects comparable to those of shorter shocks. However, there are conditions in which closing (C) and opening (O) effects of the current become operative. As illustrated schematically by line II of figure 2, when *C* occurs during the refractory period and *O* during the vulnerable period, *O* causes a premature systole or fibrillation, depending on the intensity of the current. If, as illustrated, by line II, A, *C* occurs during the vulnerable period, *C* produces similar effects when currents are strong. In the case of weaker currents which only cause a *C* premature beat, *O* has no effect because it falls during the refractory phase of the premature beat. With still weaker currents, *C* causes no effect during the vulnerable period, but if *O* falls during early diastole, premature contraction results. When *C* and *O* of such a current occur exclusively in diastole, as illustrated in line II, B of figure 2, only a premature *C* contraction is induced.

The curves of figure 4 illustrate the effects of 17 M.A. currents, 0.05 second in duration, when they occur progressively earlier in the natural heart cycles. Shocks 1 and 2, in early diastole, cause a feeble premature beat as does shock 3, which is placed in the vulnerable period. Shock 4 falls a

[4] It will be noted that the natural E.C.G. waves exhibit a pronounced monophasic tendency, which developed suddenly after one of the preceding defibrillations. While this did not affect the results, we have chosen this record for the lesson it teaches. During the course of the observations, the notation was made that the E.C.G. suggested a posterior injury. At autopsy, it was discovered that the diaphragmatic wall of the heart had been burned by the unprotected defibrillating electrode. These were employed in our earlier experiments since no harmful effect was discovered. In this case, however, the edge had apparently come into contact with the heart, causing the burn. Subsequently we employed only padded electrodes soaked in saline and suggest this precaution to those who have occasion to use such electrodes.



Figs. 3–6

little later and produces fibrillation. In figure 5, shocks 1 and 2 of 15 M.A. and 0.05 second in duration fall during the vulnerable period and produce a single premature contraction; shock 3, which falls during a similar period and has the same duration but a strength of 17 M.A., causes fibrillation.

Figure 6 shows that strong shocks (e.g., 15 M.A., 0.05 sec. in duration) occasionally elicit still other reactions, not seen with briefer ones. Shock 1 falls during the refractory period and is ineffective. In shock 2, *C* is too early to be effective; and *O* produces a premature systole. In shock 3, *C* occurs during the vulnerable phase and *O* in early diastole. The immediate effect is an artificial premature beat *X* which causes an insignificant pressure elevation. This, however, is followed later by another spontaneous[5] premature beat *Y* which, to judge from the E.C.G., arises from a different focus. Shock 4 falls entirely during the vulnerable phase and produces the anticipated fibrillation.

III. Currents which have a minimal duration of 0.10 to 0.14 second may cause fibrillation when *C* or *O* occurs during the vulnerable period. In addition, they have the inherent possibility that both *C* and *O* of the current may cause excitation during non-refractory periods.

As illustrated in figure 2, line III, when *C* occurs during the refractory period and *O* during the vulnerable period, the latter causes fibrillation when the current is strong, otherwise a premature contraction. However, this may be followed by another spontaneous contraction. If, as illustrated in figure 2, line III, A, *C* occurs early in systole and *O* during early diastole, several results may appear: 1, A spontaneous beat[5] may occur while the current remains closed; 2, *O* may induce a premature beat or 3, both may occur. In the latter case when *O* falls during the vulnerable period of the spontaneous premature beat, fibrillation may result. We can therefore understand why *O* of a prolonged direct current extending into diastole can cause fibrillation. If the relation between the duration of the D.C. shock to the incidence or duration of the premature contraction alters ever so little, fibrillation does not occur. Thus, if *O* falls during its refractory phase, it has no effect; if it falls during relaxation, another premature beat results.

If, as exemplified in figure 2, line III, B, a direct current 0.10–0.14 second in duration is applied during diastole, *C* causes a premature beat and the effects of *O* depend on its incidence during the refractory, vulnerable, or diastolic phase of the *C* premature beat. In other words, such currents may cause one premature beat, fibrillation, or two premature beats. We are thus able to understand why more prolonged shocks during diastole may fibrillate, whereas brief ones cannot accomplish this. It also makes it obvious why the effects of more prolonged shocks cannot be predicted or,

[5] For lack of a better designation, we refer to premature beats as "spontaneous," when they do not definitely follow *C* or *O*.

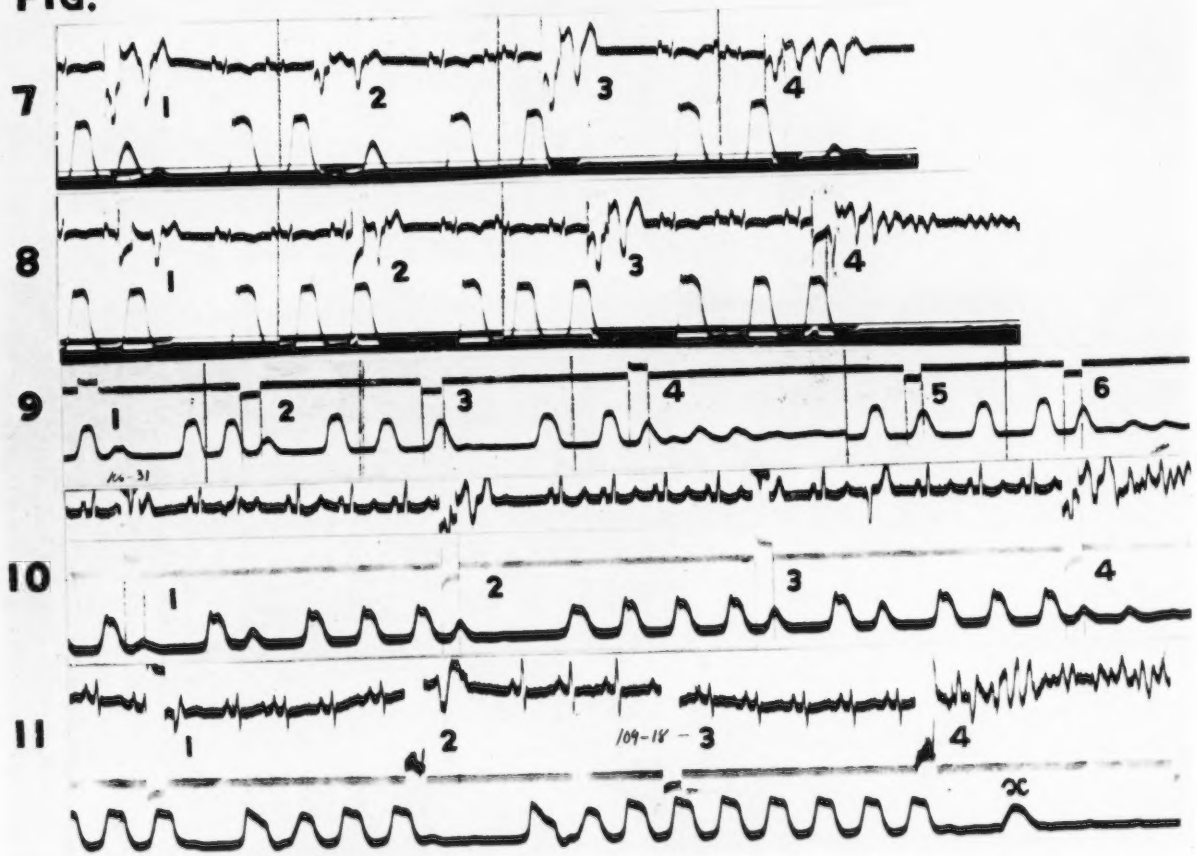
when they occur, cannot be evaluated without analytical material such as we are presenting. Finally it should be clear that such fibrillating potency disappears again when D.C. shocks are lengthened still more.

In figure 7, currents of 9 M.A. and 0.10 second duration were used throughout. In shocks 1 and 2, *C* occurs early in diastole, causing a premature beat; *O* falls during its refractory period. However a spontaneous premature beat occurs later. In shock 3, *C* occurs during the vulnerable period causing an artificial premature contraction and *O* in early diastole, a second one. Obviously, such reactions show clearly that *C* of such a strength current, when applied during the vulnerable period, is below the fibrillation threshold. But when, as in shock 4, during the vulnerable period *C* causes a premature contraction and *O* presumably falls during the vulnerable period of this premature beat, the two effects together eventuate in fibrillation.

In figure 8, from the same experiment, direct currents of 9.5 M.A. were applied for slightly varying intervals indicated on the curve. Shock 1 is followed by a single premature beat which probably arises spontaneously. Shock 2 is followed by a single contraction, more probably due to *O*. Shock 3 causes a premature *C* and a premature *O* contraction. Shock 4, which covers a large part of systole with *O* occurring in early diastole, leads to fibrillation.

In the experiment of figure 9, shocks of 5 M.A. were applied for 0.14 second so that they advanced slightly with respect to heart cycles. Shock 1 induces only an *O* premature beat. In shock 2, *C* causes a premature beat in early diastole, *O* being ineffective during the refractory phase. In shock 3, *C* causes a large premature beat later in diastole while *O*, falling in the vulnerable phase of this beat, causes another small premature beat. In shock 4, *C* similarly causes a premature beat and *O*, presumably falling in the vulnerable phase of this beat, causes fibrillation. Similar reactions to long diastolic shocks are shown in beats 5 and 6.

A glance at the pressure waves of premature beats following *C* shocks 1, 2, 3 and 4 reëmphasizes that the coincidence of an *O* shock with the vulnerable period of these beats depends not solely upon the time difference between *C* and *O*, but also upon the latency of the premature beat and the time that its summit is reached. Both are conditioned by the time of diastole in which a premature beat is induced. (For details cf. Wiggers (4).) However, the fact must be faced squarely that we cannot relate the vulnerable phase of premature ventricular beats as definitely to ventricular pressure curves as is possible in normal beats. This is illustrated by records of figure 10 in which currents of 9 M.A. and 0.12 second in duration were opened at the summit of a *C* premature beat in shocks 1, 2, 3 and 4. In shocks 1 and 3, *O* was ineffective; in shock 2, *O* caused another premature beat and in shock 4, *O* led to fibrillation. In other words, the heart was



Figs. 7–11

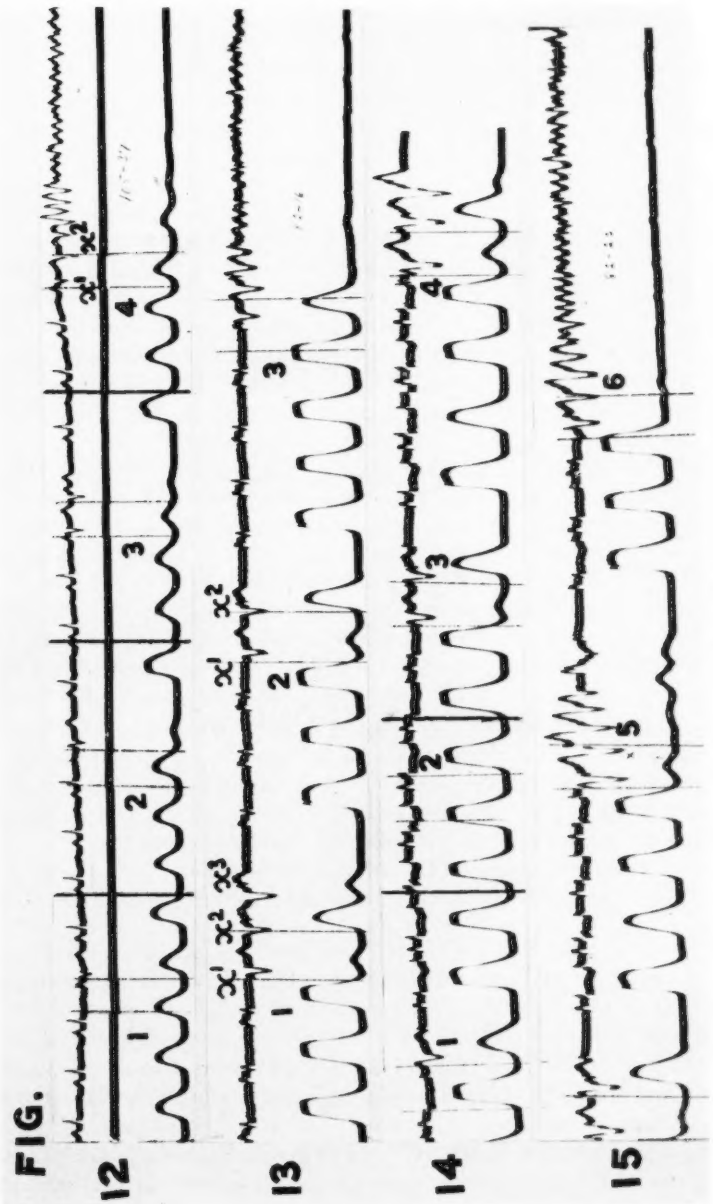
respectively refractory, vulnerable and reactive to diastolic stimuli at the summit of a pressure curve. This is not surprising in view of the fact that with aberrant excitation involved in premature beats, the ventricular pressure curve represents the resultant of many fractionate contractions which neither start nor end in phase. Consequently, the summit bears a very variable relation to the vulnerable phase of the fibers at any point selected for stimulation.

Furthermore, there is a great deal of variability in relation to the amplitudes of E.C.G. deflections and pressure variations in premature beats evoked under different circumstances. This is well illustrated in various records presented, e.g., figure 3, 1 and 4; figure 6, 3, *X* and 3, *Y*; and figure 7, 1 and 2. The pressure variation that occurs during the initial stages of fibrillation shows even more variability in relation to the E.C.G. deflections. As a rule, they show very small pressure variations, as illustrated in figure 3, *5*; figure 4, *4*; figure 5, *3*; figure 6, *4*. Occasionally a number of definite elevations occur giving the erroneous impression of coördinated beats, belied by the E.C.G., as was the case in figure 10, *4*. To this may be added figure 11, *4*, in which fibrillation caused a most unusual pressure variation *x*, while the E.C.G. was totally irregular.

These difficulties in defining any vulnerable state of premature contractions is discussed at length in order to emphasize the difficulty of presenting crucial proof for our probable interpretation that direct currents applied during diastole can only cause fibrillation when *O* falls during the vulnerable period of a premature beat induced by *C* or arising spontaneously during passage of the current.

IV. Direct currents longer than 0.20 second may exert many of the actions of currents with shorter durations. In addition, they may cause fibrillation at current intensities that are very small. Figure 12 shows a record in which direct currents of only 1 M.A. were applied at different temporal relations to heart cycles for 0.25 second each. Closure of such feeble currents during the vulnerable period is ineffective. Stimuli 1 and 2 show that *O* during diastole causes a premature beat. In beat 3, *C* and *O* during diastole each give a premature response. A similar incidence of *C* and *O* stimuli at 4 leads to fibrillation, however. An examination of the E.C.G. shows clearly that the first premature systole x^1 is doubtless artificial and due to *C*; the second x^2, however, starts before *O* and is therefore probably spontaneous. If it be assumed that this spontaneous premature impulse comes during the vulnerable period of the first, the fibrillation is accounted for in a logical manner.

In figure 13, a direct current of about 1 M.A. was periodically applied for 0.33 second. Stimulus 2 causes at *C* an artificial premature beat x^1 plus a spontaneous beat x^2. Stimulus 1 reacted likewise but, in addition, evoked another spontaneous beat x^3 some time after *O*. These records



Figs. 12-15

illustrate that the tendency of the myocardium to develop spontaneous beats increases as the duration of the stimulus increases. With long applications, e.g., 1 second, it may evoke a continuous series of such beats and, if opening falls at a fortuitous moment, fibrillation results. Fibrillation also results when a spontaneous beat occurs after *O* at the vulnerable period of an *O* premature beat. Stimulus 3 was fortuitously so applied that *C* and *O* fell precisely in vulnerable periods. It is improbable that the *C* shock of 1 M.A. could itself have induced fibrillation; but when *O* comes during a vulnerable phase of the premature beat it elicits fibrillation. This and similar observations make it probable that the fibrillating threshold of aberrant beats is lower than that of normal beats; but the question requires more thorough study.

Figures 14 and 15 show compact illustrations of the different effects which can be anticipated from a 6 volt current, 0.33 second in duration. These are: No effect (stimulus 2); one premature systole (stimulus 1); two premature systoles (stimulus 3); three premature beats (stimulus 4); a run of premature beats (stimulus 5) and fibrillation (stimulus 6). In the last event, closure falls during the vulnerable period, but it is unbelievable that closure of such a weak current alone was responsible for the fibrillation.

A note on chronaxie of the ventricles. It is not our purpose to discuss in detail the validity of chronaxie determinations of the mammalian ventricle. However, our observations clearly indicate the inherent difficulty of determining a rheobase, for a direct current over 0.05 second in duration can cause a multitude of effects, and if applied at appropriate moments, may even fibrillate the ventricles. Attempts to establish a rheobase in the beating heart by using the development of premature beats as a criterion, without regard to the incidence of *C* and *O* of such currents and without evidence as to whether such beats are effects of *C* and *O*, or arise spontaneously, seem to us a valueless procedure. This, together with the fact that the beating heart, unlike nerve or resting skeletal muscle, has no span of iso-excitability during any part of the cycle, seems to invalidate present methods for determining chronaxie as a measure of ventricular excitability.

SUMMARY AND CONCLUSIONS

This research was designed to establish, as far as possible, the conditions under which ventricular fibrillation is produced by direct currents of different durations and to offer a logical explanation for its occurrence. For this purpose D.C. shocks ranging from 1 to 50 M.A. were applied for intervals of 0.01 to 0.33 second to a small area of dogs' left ventricles by nonpolarizable electrodes. Shocks were introduced in alternating directions at every 6th ventricular beat and were so spaced that they fell progressively earlier or later in relation to normal cycles. In this way, the incidence of closing and opening as well as duration of action could be established. Our analysis leads to the following conclusions:

1. The dominant factor which determines the induction of ventricular fibrillation by any electrical stimulus is the fact that any type of stimulus above a certain critical value, introduced during the vulnerable period of late systole, causes simultaneously a premature contraction plus some local or generalized disturbance of conduction which permits irregular reëntry of impulses and leads to the various stages of fibrillation described by one of us (5).

2. In the case of direct currents, the effective excitant may be (a) a brief rectilinear shock, probably not more than 0.04 second in duration or (b) the closing or opening of more prolonged currents during this period of vulnerability.

3. In the case of D.C. stimuli which exceed 0.05 or 0.06 second, several secondary factors may enter which also lead to fibrillation.

The factor which determines fibrillation in any specific instance depends upon the duration of the D.C. stimulus, upon the time that closure occurs with respect to the cycle, upon the character of the premature response evoked, upon the relation of opening to a normal or premature beat, etc. However, we believe that it can be demonstrated in many of these highly variable conditions, and is probable in others which cannot be so definitely analyzed, *that a second effective stimulus, artificial or physiological*, must strike during the vulnerable period of a premature systole in order to cause fibrillation. Such a concept harmonizes the apparent discrepancy that brief shocks of any form only cause fibrillation when they are applied during the vulnerable period, whereas D.C. shocks longer than 0.05 second may do so, when they fall entirely in diastole, partly in systole and partly in diastole, or even when they extend over several beats.

4. The chief secondary mechanisms by which shocks longer than 0.05 or 0.06 second become more potent fibrillation stimuli, are fundamentally due to two well known peculiarities of such shocks: 1. They are no longer unitary stimuli but are capable of exerting separate *C* and *O* effects, and 2. they cause effects (electrotonic?) during persistence of the current, which give rise to unpredictable spontaneous impulses either during the flow of the current or shortly after its cessation.

5. The manner in which such secondary phenomena lead to fibrillation with currents of increasing duration (0.07–0.33 sec.) is graphically depicted in figure 2. In a broad way, these conditions can be resummarized verbally as follows:

(a) When *C*, falling during the vulnerable phase or early diastole, causes a premature contraction of such duration that *O* of effective strength occurs during its vulnerable period.

(b) When *C* occurs during the refractory period of a normal cycle, but the continued passage of the current causes a spontaneous premature beat in the next or any subsequent diastole and *O* occurs during its vulnerable period.

(c) When *C* occurs during the refractory period of a normal cycle and *O* falls during early diastole of this or any subsequent beat causing a premature contraction *and* later a spontaneous premature impulse which coincides with the vulnerable period of the first premature beat due to *O*.

6. In view of (a) the greater tendency even of weak currents, 0.24–0.33 second in duration, to evoke spontaneous premature beats not related to *C* or *O*; (b) the frequency of fibrillation by such currents in our series and (c) the non-existence in the beating heart of any span of iso-excitability, the determination of chronaxie of the ventricles by use of direct currents introduces difficulties.

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PRODUCTION OF VENTRICULAR FIBRILLATION BY ALTERNATING CURRENTS[1]

RENÉ WÉGRIA[2] AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University, Medical School, Cleveland, O.

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In the preceding paper, we analyzed the effects of localized direct currents of different durations upon the dog's ventricle, with special reference to the fundamental conditions under which they produce fibrillation. This communication deals with a similar analysis of the effects of alternating currents, based on many observations upon 21 dogs.

The anesthesia, operation, method of defibrillation and experimental procedures were the same, except that an electromagnetic key operated by the cylinder contact-breaker, *R*, needed to be substituted for the gas triode, *T*, of figure 1 in the previous paper. While 60/second sine waves probably do not constitute the most effective stimuli, our studies were largely restricted to such a frequency, owing to the practical relation of such currents to production of fibrillation. The currents employed were either a house current, reduced to proper voltage by means of a rheostat, or that produced by a G.R., type 377, oscillator. Left intraventricular pressure, a standard E.C.G. and an oscillographic tracing of the A.C. stimulus constituted our routine recordings. Unfortunately, in the great reduction of records, the sine waves are not always reproduced clearly but the onset and offset are clearly marked. One or the other of these records is occasionally omitted in reproduced curves to conserve space.

RESULTS. Although the conditions under which 60 cycles alternating currents cause fibrillation are very similar to those under which direct currents are effective, it is important that these facts be critically established. In addition, the study of such records brings out many significant observations which must be added to our stock of facts upon which a theory of ventricular fibrillation may ultimately be based.

Stimuli composed of $\frac{1}{2}$ to 4 waves (0.0083 to 0.0666 sec.), like brief D.C. shocks, act as a unitary stimulus, for while each wave is a potential stimulus, only one can be effective, since the waves following the effective one

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[2] Fellow of the C. R. B. Educational Foundation.

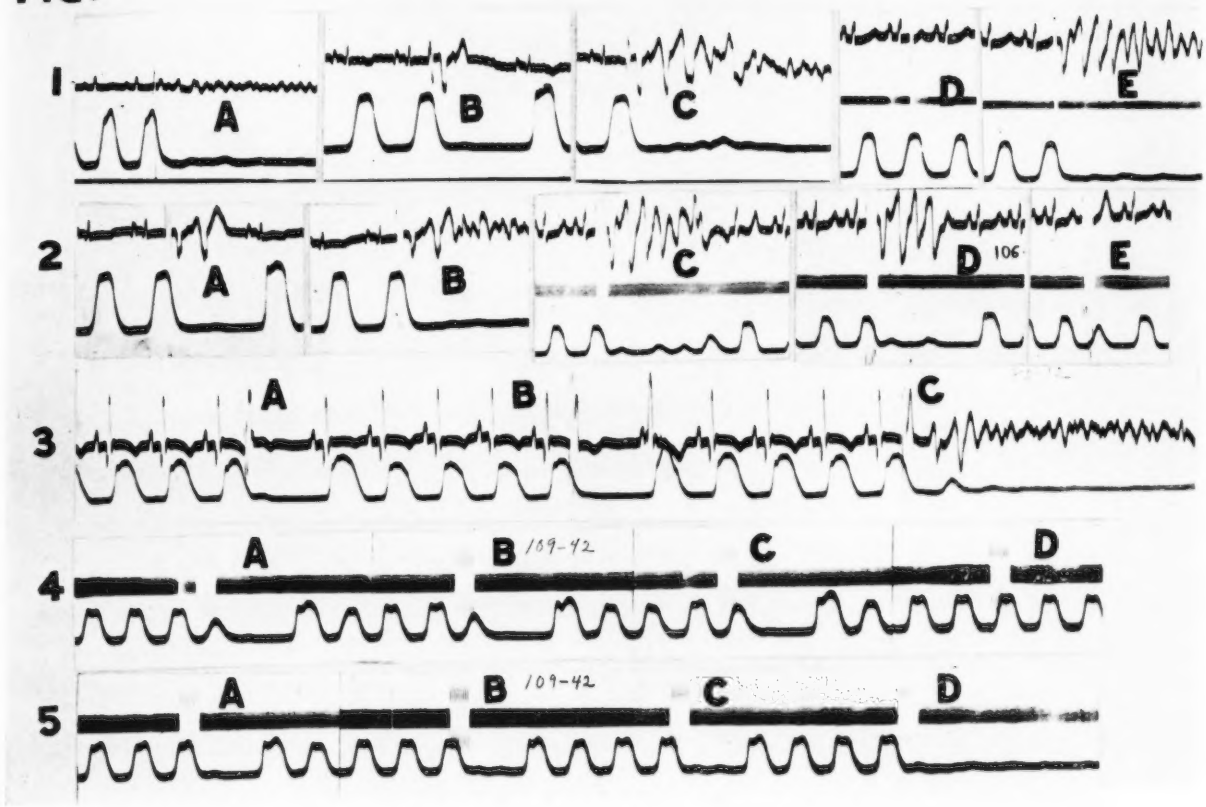
fall during the refractory period of any premature beat evoked. Generally this can be extended to A.C. stimuli of 5 or 6 sine waves; but under exceptional conditions these may evoke a double response, conveniently discussed with somewhat longer shocks (see below).

Even one-half of a 60 cycle sine wave—the shortest attainable with our apparatus—causes all the effects of 3 or 4 such waves. As shown in figure 1, A, if strong enough and applied during the vulnerable phase of late systole, it causes fibrillation. However, such partial waves, like one whole wave, may, at critical voltages, produce either a premature beat, as in 1, B, or fibrillation as in 1, C. The possibility exists that this is due to the phase angle at which the stimulus begins, a question that we were not in a position to investigate thoroughly. A glance at the original records shows a phase start at approximately 0° in 1, B, and about 220° in 1, C.

Two or three sine waves of sufficient voltage cause fibrillation when they fall strictly within the vulnerable period or start in this period, as shown in figure 1, E. During the refractory phase, as shown in figure 1, D, they evoke no response. In these, as in experiments with brief condenser and D.C. shocks, it is extremely difficult to ascertain the exact span of the vulnerable period. For example, we have found occasionally that a stimulus applied during early diastolic relaxation causes fibrillation and we have periodically been inclined to include this in the vulnerable phase. The records of figure 2, A and B, are interesting in showing that this is not universally true, or at least that the last moments of systole seem to be more highly vulnerable. In both cases, 3 sine waves of the same strength fall toward the ends of vulnerable periods, but sufficiently later in 2, A to overlap slightly into the phase of isometric relaxation. Fibrillation occurs only in 2, B. Such observations become significant only when repeated. Therefore, figure 3 is also presented, for it shows beautifully the clear demarcation of the vulnerable phase at the moment when isometric relaxation occurs. In figure 3, A, B, C, three sine waves fall ever so slightly earlier in the cycle; but only the last stimulus which actually starts during late systole induces fibrillation.

As the number of waves increases, the chance of diastolic overlap becomes greater. In figure 2, C and D, five waves falling partly in the vulnerable phase and partly in early diastole give rise to a series of reentrant waves of excitation shown in the E.C.G.; whereas the same series falling solely in diastole evoke a single premature beat only (fig. 2, E). Such a series of reëntrant waves represents a potential fibrillation. They are very like those which initiate fibrillation, e.g., as illustrated again in figures 1, C, 1, E, 2, B and 2, C. Why fibrillation does not eventuate must be studied further, but it stresses the fact that any satisfactory theory of fibrillation must explain *a*, the initiation of a premature contraction and a series of reëntrant waves, and *b*, the factors which favor their transformation into true fibrillation.

FIG.

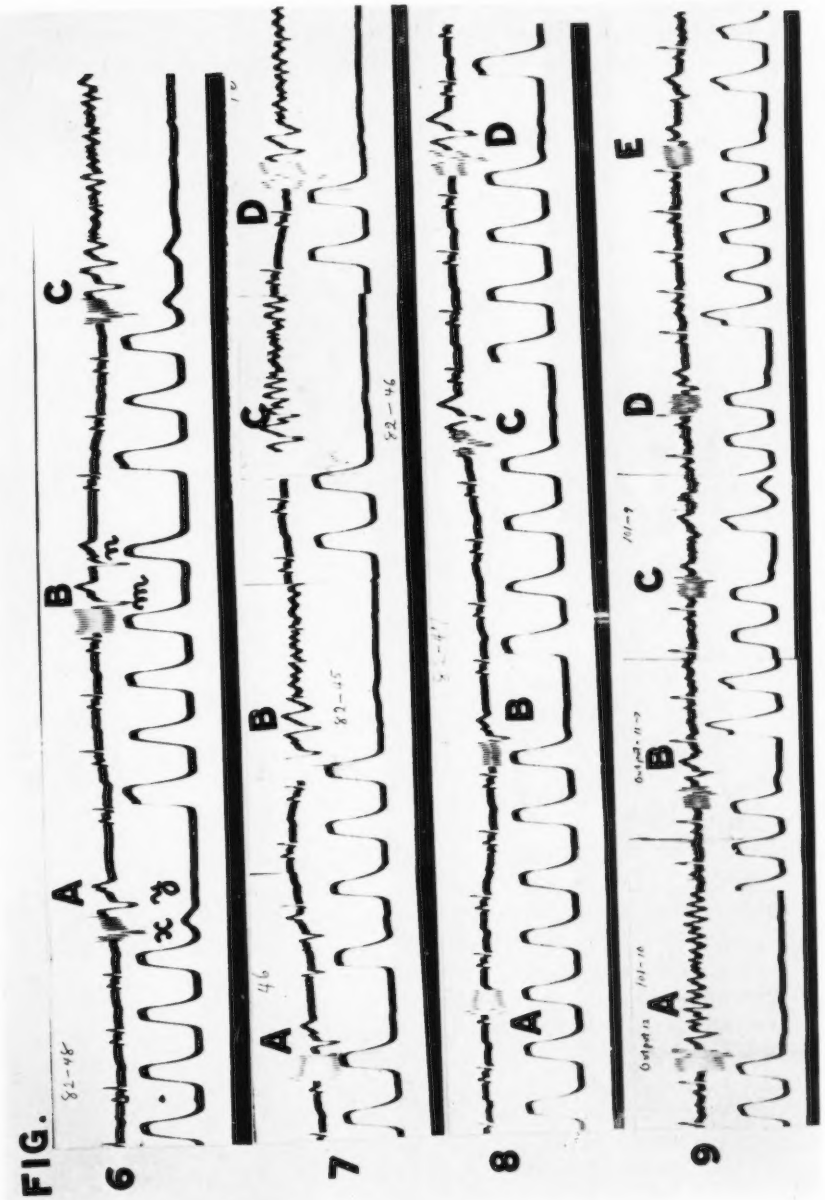


Figs. 1-5

A.C. stimuli containing 7 to 9 60/second sine waves (0.116 to 0.15 sec.) produce a variety of effects depending upon their strength and placement in the cardiac cycle. When they are very weak, they are without effect or produce an occasional premature contraction. In figures 4 and 5, a series of 9 larger waves falling frankly in diastole (fig. 4, A, B, C) evoke two premature contractions, the second of which is barely perceptible in original ventricular pressure curves. This causes an extension of the compensatory pause by the time-equivalent of one normal beat, i.e., interpolation of two premature beats during a normal compensatory pause does not occur. When the waves fall entirely during the refractory period (fig. 4, D), they are ineffective; but as soon as they encroach upon (fig. 5, A), or start in (fig. 5, B and C) the vulnerable phase, they evoke a single premature contraction followed by a compensatory pause. It is important to note that currents of such strength did not cause fibrillation through action during the vulnerable period of late systole. However, if they start ever so early in diastole, as in 5, D, fibrillation results. We may emphasize that with an appropriate number of waves, weaker currents which cannot induce a *systolic type* of fibrillation can cause a *diastolic type*. Of course, if the currents are stronger and fall anywhere in the vulnerable period, they do cause *systolic fibrillation*.

The reason that A.C. stimuli are effective in causing "diastolic fibrillation" is probably found in the fact analyzed in connection with D.C. shocks, viz., that the first of these sine waves is not strong enough to cause such a premature beat when applied during the vulnerable phase, but falling during the isometric relaxation is adequate to inaugurate a premature beat. The train of waves is then long enough so that an effective portion falls during the vulnerable phase of this premature beat. In short, the induction of diastolic fibrillation is determined, as in the case of direct currents, by *a*, the start of the wave series with respect to the cycle; *b*, the character of the premature response evoked, and *c*, the relation of an effective portion of such a series to the vulnerable period of a premature beat. In the case of a D.C. shock, this would need to be the opening of the current; in the case of A.C. currents, any phasic change might suffice, thereby increasing the chance of inducing fibrillation by such currents.

How precisely the series of weaker shocks must be placed with reference to a cardiac cycle in order to cause diastolic fibrillation is illustrated in figure 6 in which 11 waves were applied. In A, it merely evokes two premature beats, *x*, *y*, and in C is followed by fibrillation. The superimposed stimuli and E.C.G. of figure 6 show clearly that the effective moment of a series of sine waves which elicits the premature beat—regardless of whether it is followed by reëntry beats and fibrillation—may be the onset of such a series as in figure 6, A and C. However, if a reasonable latency be allowed in figure 6, B, it is apparent that one of the intermediate waves must have



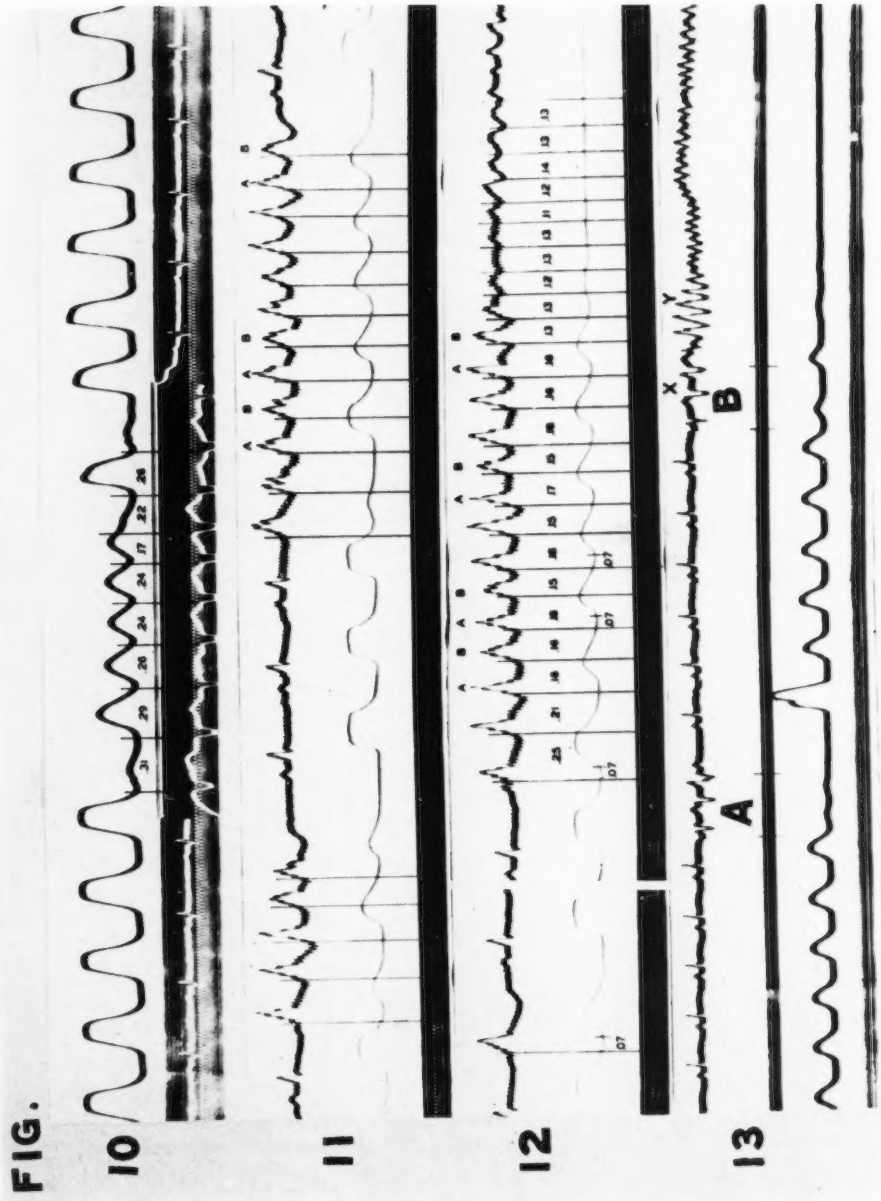
Figs. 6–9

caused the premature systole, *m*. The second one, *n*, on the other hand, appears to be spontaneous in origin. An examination of many curves permits the inference that moderate alternating currents exceeding 0.16 sec. in duration can cause changes leading to such after-effects, much as do direct currents.

A clear instance in which strength of the current is of moment in the production of systolic fibrillation by a series of 10 or more waves is presented by curves of figures 7 and 8 from the same animal. In each case, 11 sine waves were applied. Those of figure 7 had a strength represented by an output of "12" on the G.R. oscillator and those of figure 8, an output of "10." In the former record, systolic fibrillation occurs when the series of waves extend through the vulnerable period (fig. 7, B), when they start in the vulnerable period (fig. 7, C), but not when they end there (fig. 7, A). With weaker currents, only premature systoles occur as in figure 8, B, C and D; while it is ineffective in 8, A. In addition, figure 7, A gives evidence, by a spontaneous premature beat, of some persistent after-effect. The curves of figure 9 show another example in which a series of waves with an output of "12" on the G.R. oscillator causes systolic fibrillation when they cover the vulnerable period (fig. 9, A); whereas a similar series of waves with an output of "11" fails to do so during any phase of systole (fig. 9, B, C, D and E). Consequently, were it not for the fact that a series of weaker sine currents may induce diastolic fibrillation when effective portions strike at appropriate moments of normal and premature beats, the strength of current composed of 10 or 15 waves (ca, 0.25 sec.) might be used to evaluate the sensitivity of the ventricle to fibrillation.

The effects of more prolonged but very weak alternating currents bring to light phenomena which are obscured by the prompt induction of fibrillation in the case of stronger currents. Weak A.C. stimuli (0.5 M.A. to 1 M.A.), when their duration exceeds 0.2 second, evoke a series of aberrant beats shown in figures 10 to 13. While the development of such a ventricular tachycardia by currents of several seconds' duration is well known to experimenters, the reactions have never been fully analyzed, particularly with reference to their transition into fibrillation.

A detailed study of original records, a few samples of which are shown in figures 10, 11, 12 and 13, reveal the following additional details: The tachycardia starts almost immediately after application of weak A.C. The frequency of beats calculated from E.C.G. deflections ranges from 260 to 375 per minute. It is slower than that of the tachysystolic waves (600 per min.) which are introductory to true fibrillation (cf. fig. 13, *X* and *Y*). The frequency is remarkably constant in the same heart, stimulated at the same points. Each electrical deflection is accompanied by a ventricular pressure wave when the frequency is between 250 and 280 (fig. 10); but when the E.C.G. deflections exceed a critical rate, somewhere between 275 to 300 per



Figs. 10-13

minute, the ventricular pressure waves show an alternate lapse (figs. 11, 12). Such dissociation of mechanical and electrical systoles has apparently not been previously described. The spacing of the electrical deflections is rarely even and the corresponding ventricular beats may therefore be variable in amplitude (fig. 10). Different curves have different characteristics. As a rule, the period of the first deflection is definitely longer than that of following deflections. Often a progressive decrease in duration occurs for a few deflections, after which they become more evenly spaced (fig. 11). However, irregularities creep in. Sometimes the electrical deflections are alternately a trifle longer and shorter, and occasionally as shown in beats labeled A and B in figures 11 and 12, the electrical deflections seem to alternate slightly in amplitude. In such cases, the larger deflection is preceded by a shorter cycle and is the one which accompanies a ventricular pressure wave. The ventricular tachycardia induced by very weak A.C. may revert to a normal rhythm shortly after cessation of the stimulation, as in figures 10, 11, 13, A; or it may be converted to fibrillation *either* while current continues to be applied (fig. 12), or shortly after its removal (fig. 13, B). The transition is fairly distinct in either case. It consists in a sudden reduction in the periods of the aberrant E.C.G. waves, e.g., in figure 12 from 0.17 to 0.13 second and in figure 13, B from 0.18 to 0.10 second, i.e., to rates of 460/min. and 600/min., respectively. These beats correspond to the initial stage of fibrillation designated as tachysystolic by one of us. They lead irrevocably into the subsequent transitional stages of true fibrillation.

The question arises, is the tachycardia which occurs during application of weak currents due to release of repetitive stimuli from a focus—presumably in the vicinity of the electrodes—, or to reëntry of impulses? Somewhat in favor of the latter is the fact that an alternate default of left ventricular pressure waves is difficult to explain by repetitive focal stimuli released near electrodes *placed on the left ventricle*. The progressive shortening in periods of E.C.G. deflections is consistently explained by formation of shorter paths of reëntry or progressive increase in conduction rate. However, it can also be explained on the theory of focal stimulation if we assume that passage of A.C. causes a decrease in refractory periods. A certain variability in refractory periods would account for random variability in spacing. Consequently, while consideration of our results favors the concept of reëntry, conclusive evidence cannot be claimed for such a theory.

Why does ventricular tachycardia eventuate in fibrillation in some instances and not in others? We have conclusive evidence that this is not a matter of duration of stimulation, provided the period exceeds only 0.2 sec. (cf. fig. 13, A and B). The mechanisms concerned remain obscure; any ideas are purely speculative. Two speculations may be briefly considered as to probability:

1. In accordance with our general concepts, fibrillation occurs when an effective portion of a stimulus falls during a vulnerable period. The vulnerability may be so enhanced by passage of a weak current that a portion of the series becomes of fibrillating potency. This could not explain fibrillation which follows removal of the current. Again, a natural or ectopic impulse arising at some portion of a reëntrant wave during the local vulnerable period might be strong enough to set off the fibrillation. Careful study fails to reveal convincing evidence of such a possibility.

2. The fibrillation may be unrelated to the focal point of stimulation. Assuming that a series of reëntrant waves occurs over slightly different pathways, the possibility exists that shorter circuits are suddenly formed due to localized variation in refractory periods which lead to the initial tachysystolic phase of fibrillation. Between these and still other speculations, no decision is possible on the basis of available experimental evidence.

SUMMARY

The effects of 60 cycle alternating currents applied locally to the dog's left ventricle through nonpolarizable electrodes are analyzed:

A.C. stimuli composed of $\frac{1}{2}$ to 4 waves (0.008–0.0666 sec.), like brief D.C. shocks, act as a unitary stimulus. When they start or fall entirely within the vulnerable period, they always give a response if strong enough; that response is one or two premature ventricular systoles followed or not by ventricular fibrillation. The influence of the moment of onset or phase angle in relation to the vulnerable period has not been established. A similar stimulus during the refractory period is ineffective. When given during diastole, it causes one premature beat and never produces fibrillation.

Stimuli composed of more sine waves (generally 7 to 9) produce no effect or an occasional premature beat when they are very weak. A similar number, of moderate strength, falling entirely in the refractory phase are ineffective; but when they encroach upon, cover, or start in the vulnerable period, they evoke a single premature contraction, but never cause fibrillation. If they start ever so early in diastole, however, fibrillation occurs. Strong currents also cause fibrillation when they enter during the vulnerable period.

The occurrence of fibrillation by early diastolic shocks, incapable of acting during the vulnerable period, can be explained by the fact that an effective portion of the series now falls during the vulnerable period of a premature beat. In other words, an apparent diastolic fibrillation is actually a fibrillation started during the systolic vulnerable period of a premature beat.

A.C. stimuli, with durations varying from 0.2 to 1 second or more, promptly cause fibrillation when currents are of moderate or great strength.

However, even very weak currents (0.5 to 1.0 M.A.) cause ventricular tachycardia which may revert to a normal rhythm after removal of the stimulus or may eventuate in fibrillation either while the A.C. is operating or shortly after its withdrawal.

An analysis of the ventricular tachycardia reveals that the rhythm is not quite regular and that a tendency to progressive increase in rate occurs. In some instances, electrical alternation exists and a peculiar dissociation of electrical and left ventricular pressure occurs in alternate beats, despite the fact that electrodes are applied to the left ventricle. Therefore, the probability is weighed that the tachycardia is caused by reëntry rather than by periodic focal stimuli. However, conclusive evidence for such a theory is not adduced.

The reason why weak alternating currents produce fibrillation in some tests and not in others remains obscure. However, definite evidence is presented that the duration of such currents is not a factor provided this exceeds 0.2 sec. The mechanisms through which fibrillation eventuates during or shortly after use of such weak A.C. currents are difficult to analyze on the basis of evidence so far available.

THYROID THERAPY AND THE SKULL BONE PATTERN OF THYROPARATHYROIDECTOMIZED RATS

MARY C. PATRAS AND G. E. WAKERLIN

From the Departments of Physiology of the University of Illinois and Loyola University School of Medicine

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The functions of thyroid and parathyroid tissue have been extensively studied by many investigators in relation to metabolism, general growth, development of systems and the chemical equilibria in tissues. The problems have been approached by extirpation of the glands separately and together, and by feeding or injecting glandular preparations.

Simpson (1) called attention to the importance of age at the time of thyroidectomy or parathyroidectomy in determining the severity of the deficiency symptoms. He described typical cretins resulting from thyroidectomy at a very young age as being small and stunted animals with broad faces and rickety limbs. Later Basinger (2) described the short extremities of thyroidectomized rabbits as "pseudorickets". That young animals are more susceptible to parathyroidectomy than older ones has been frequently confirmed (3, 5).

The rôle of diet (3, 5) following extirpation of the parathyroid glands has been extensively studied. Patras *et al.* (4) observed that the nutritional condition of the animal in relation to the mineral content of the diet prior to thyroparathyroidectomy, as well as immediately following the operation, contributed to the character of the convalescence.

The importance of environmental temperature (6) and intercurrent disease such as rickets (7), infections, constipation (8) and gastro-intestinal irritation (9) in precipitating tetany from a latent state has been suggested.

Voluminous evidence has been submitted to prove the importance of the parathyroid glands in the development of the skeletal system. Hammett (10) found the bones of parathyroidectomized animals to be low in ash, calcium and phosphorus; and deficiency findings in the teeth have been observed by many investigators (3, 5). Marked decalcification (osteoporosis) has often been observed in clinical hyperparathyroidism and frequently has been produced experimentally by the injection of toxic doses of parathyroid extract (5).

The skeleton is composed of constituents which by the processes of

metabolism are made mobile. Inorganic elements laid down at one time are replaced by incoming elements at a later time. Thus the continuous exchange of inorganic constituents between organized bone and body fluids makes the normal skeletal system an excellent storehouse for emergencies.

The extent to which the detailed patterns of normal bone structure may be altered by the hormones of the thyroid and parathyroid glands is a subject of further investigation together with the relation of this normal pattern to calcium metabolism. Patras, Templeton and Hummon (11) have described a marked disturbance in the mosaic pattern of the skull bones of albino rats following thyroparathyroidectomy. Since the growth of thyroparathyroidectomized animals can be practically restored to normal by the ingestion of desiccated thyroid it seems reasonable to assume that such post-operative treatment might restore the normal bone patterns.

The quantity of thyroid to be used was selected for this work as a result of preliminary experiments in which the growth of thyroparathyroidectomized animals receiving 0.02, 0.05 and 0.1 per cent thyroid respectively in the diets was compared with that of the normal growth of unoperated animals. From this preliminary experiment it was revealed that the group of animals receiving 0.1 per cent thyroid after thyroparathyroidectomy had a high mortality between 100 and 150 days after the start of the experiment. During this period there was a loss of weight in the surviving animals indicating a toxic state. The groups receiving 0.02 and 0.05 per cent thyroid gave growth curves practically identical, and comparable to the growth curve of the normal unoperated animals. Since 0.05 per cent thyroid did not seem more beneficial to growth than 0.02 per cent it seemed advisable to select the smaller concentration for a study of the influence of thyroid therapy on the skull pattern.

For our study on the influence of desiccated thyroid on the mosaic pattern of the skull bones we used 108 albino rats. All animals were taken from the Loyola University stock colony which is maintained on Fox Chow *ad libitum*, with bread and meat twice per week. The rats were weaned at the age of 21 days and thyroparathyroidectomized between the ages of 25 and 31 days. Immediately following thyroparathyroidectomy they were divided into 4 groups. Groups 1 and 2 (23 males and 25 females respectively) received a Fox Chow diet throughout the course of the experiment. Groups 3 and 4 (31 males and 29 females respectively) were given a diet consisting of Fox Chow containing 0.02 per cent desiccated thyroid. All animals were fed their respective diets for 200 days during which time they were weighed at weekly intervals. At the end of the experiment the femurs and skull bones were saved for measurements and x-ray study.

The beneficial effect of thyroid on the growth of the females (fig. 1) and

males became apparent between the 2nd and 3rd weeks of the experiment. This became more pronounced reaching a maximum 70 days after the beginning of the experiment at which time the animals approximated their mature growth. Beyond this time the same effect was maintained throughout the course of the experiment.

The second index was obtained by measuring the femurs after the animals had been sacrificed and the bones cleaned of all attached tissue (fig. 2). The average femur length was found to be 27.9 and 26.2 mm. respectively for the male and female thyroparathyroideetomized rats which did

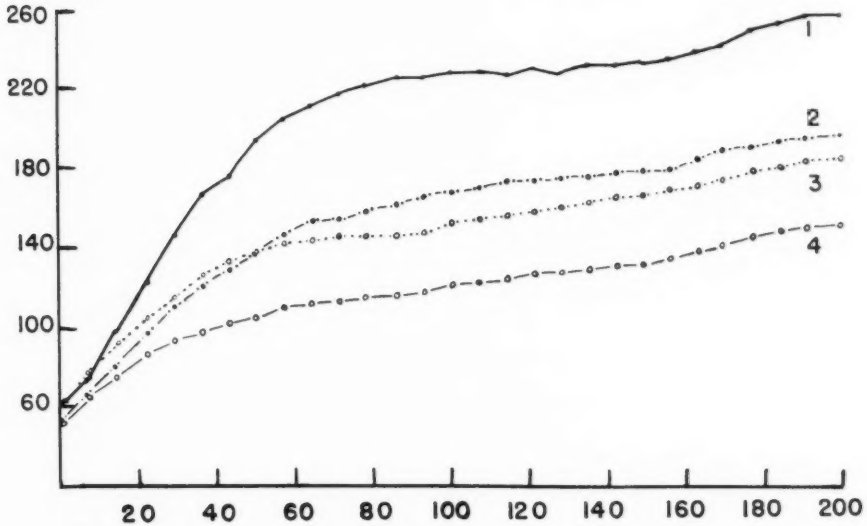

Fig. 1. Weight curve of thyroparathyroideetomized rats. Curve *1*, male rats receiving desiccated thyroid. Curve *2*, female rats receiving desiccated thyroid. Curve *3*, male rats receiving only a Fox Chow diet. Curve *4*, female rats receiving only a Fox Chow diet.

not receive thyroid in the diet. The average femur length was found to be 32.5 and 29.9 mm. respectively for the male and female animals which received a diet containing desiccated thyroid.

The roentgenogram of a normal rat's skull presents a mosaic pattern (fig. 2) which appears to be a calcified network, the interstices of which are much less dense. This normal pattern is obscured after thyroparathyroidectomy by what appears to be a decrease in the density of the network and a slight increase in the density of the interstitial areas. Careful examination of the roentgenograms of the skulls from the experimental animals revealed an increased density (fig. 2) in those groups (groups 3

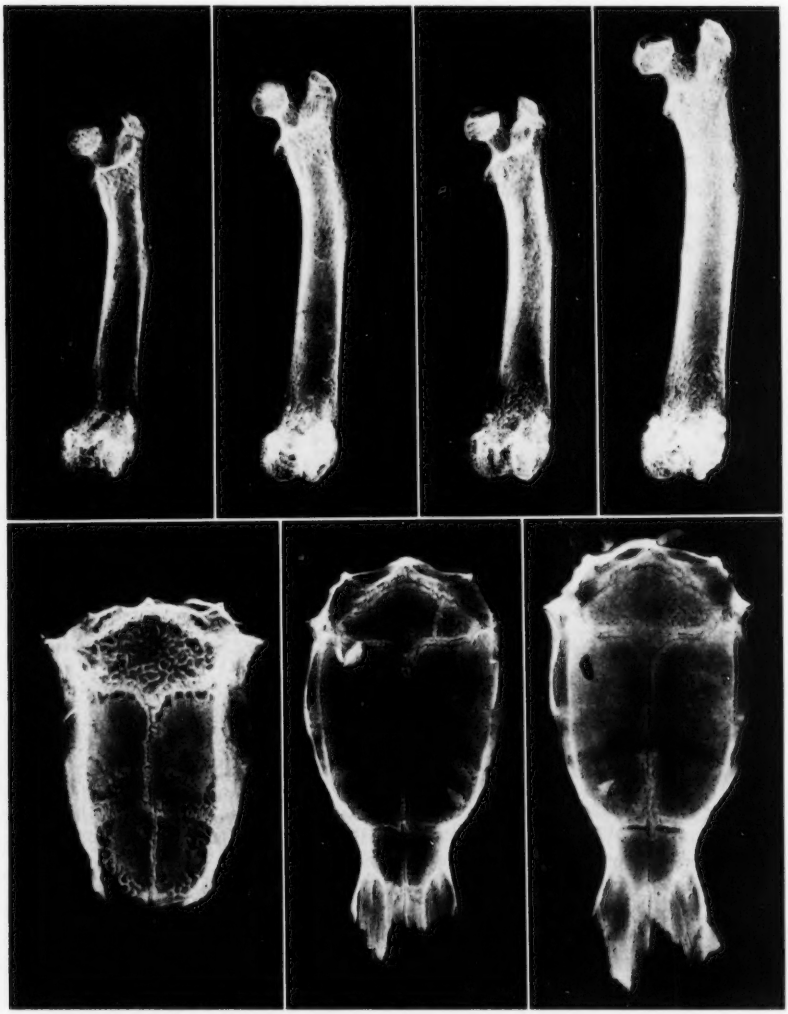

Fig. 2. Femur and skull bones. No. 1, femur of female rat on Fox Chow diet. No. 2, femur of female rat receiving thyroid in diet. No. 3, femur of male rat receiving Fox Chow. No. 4, femur of male rat receiving thyroid in diet. No. 5, skull bone from normal rat. No. 6, skull bone from thyroparathyroidectomized rat. No. 7, skull from thyroparathyroidectomized rat receiving desiccated thyroid in diet.

and 4) which received post-operative thyroid therapy as compared to those (groups 1 and 2) which did not receive thyroid. This increased

density, however, did not seem to alter the obscured details of the mosaic configuration which follows thyroparathyroidectomy.

The mosaic pattern in the thin skull bone is probably concerned with strength. In removing the bones from the animals after death, however, the skulls of those which did not receive thyroid were found to be much more fragile than those belonging to the thyroid-treated animals in spite of the fact that the pattern was not benefitted by the thyroid therapy. This seems to indicate that thyroid feeding is capable of strengthening bone by a mechanism other than the normal, namely, calcification without regard for the normal pattern. Even though the skulls of the thyroid-fed animals were harder and more dense than those of their littermates which did not receive thyroid therapy they were much more brittle and for that reason more fragile than skulls from unoperated animals.

SUMMARY

1. Preliminary experiments on thyroparathyroidectomized rats revealed that Fox Chow containing 0.1 per cent desiccated thyroid caused toxic manifestations after 100 to 150 days.

2. The ingestion of a diet of Fox Chow containing 0.02 and one containing 0.05 per cent desiccated thyroid by thyroparathyroidectomized rats was found not to cause toxic symptoms for at least 200 days.

3. A stimulating effect of 0.02 per cent thyroid in the Fox Chow diet on the growth of thyroparathyroidectomized rats, as determined by the weight curve, was observed.

4. The femur length of thyroparathyroidectomized rats was found to be increased appreciably by the ingestion of a diet containing 0.02 per cent desiccated thyroid.

5. The normal mosaic pattern of skull bones was found to be blurred following thyroparathyroidectomy.

6. The ingestion of Fox Chow diet containing 0.02 per cent desiccated thyroid was not conducive to the maintenance of a normal mosaic pattern in the skulls of thyroparathyroidectomized rats.

7. The ingestion of thyroid by thyroparathyroidectomized rats caused the development of a denser, less fragile skull in which the mosaic pattern was still blurred more than normally.

8. The skulls of thyroparathyroidectomized rats receiving thyroid therapy were more brittle and fragile than those of normal unoperated animals.

We are grateful to R. D. Templeton, R. L. Ferguson and I. F. Hummon for their coöperation in this work.

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STUDIES IN IODINE METABOLISM OF THE THYROID GLAND IN SITU BY THE USE OF RADIO-IODINE IN NORMAL SUBJECTS AND IN PATIENTS WITH VARIOUS TYPES OF GOITER[1]

JOSEPH G. HAMILTON AND MAYO H. SOLEY

From the Radiation Laboratory of the University of California, Berkeley; the Divisions of Medicine and Pharmacology of the University of California Medical School and the Thyroid Clinic of the University of California Hospital, San Francisco

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A method of studying iodine metabolism by the use of radioactive iodine in a group of patients suffering from several types of thyroid disease was described in a recent report (1). The uptake of administered iodine by the thyroid glands of these patients was determined by the measurement of the radio-iodine content of the extirpated thyroid tissue. This procedure had the disadvantages that only one measurement of the iodine uptake of the thyroid could be made on each patient and that comparable studies with normal controls could not be done. However, in a single experiment the uptake of iodine by the thyroid in situ was determined by the measurement of the gamma rays from the radio-iodine which had accumulated in the glands. By the use of this method the uptake of iodine in the thyroid glands of normal subjects and of patients can be compared and the metabolism of the accumulated iodine in these thyroids can be followed for several weeks after its administration. The present report includes a description of the results of the application of this technic to the study of iodine metabolism of the thyroid gland.

METHOD OF STUDY. The radio-iodine was prepared by the Berkeley cyclotron and converted to sodium iodide by the procedure described in a previous article (1). A solution of sodium iodide, containing 14 mgm. of iodine and an exactly known amount of radio-iodine, was administered to each subject in 100 cc. of water one hour before breakfast. The radioactivity of the radio-iodine administered in each experiment was equivalent to from 12 to 50 μgm. of radium element in equilibrium with its decay products when the gamma rays from these two substances were used as a basis of comparison.

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A scale-of-four Geiger-Müller counter was used for the measurement of the gamma rays emitted from the radio-iodine atoms taken up by the thyroid glands of patients and of normal subjects. In each case the isthmus of the gland was palpated and a small piece of adhesive tape placed over it. The counter tube employed was 10 cm. in length and 1.5 cm. in diameter and was encased for mechanical protection in a copper tube whose walls were 1 mm. thick. A mark was made at the center of the copper case so that for each measurement its position would be the same. The radioactivity of the thyroid was determined by placing the counter tube firmly against the gland. The mark denoting the center of the counter tube was placed directly against the piece of tape indicating the center of the thyroid isthmus.

The subject was given the radio-iodine solution to drink and 50 cc. of water were administered to wash all traces of the radio-iodine from the mouth and esophagus, and within five minutes the first determination of the radioactivity of the thyroid gland was made. During the following hour and a half, readings were taken every three to eight minutes. The counter tube was reset against the mark over the isthmus of the gland for each measurement. At the conclusion of the first hour and a half the subject was allowed to leave for breakfast. The determinations were resumed within the next hour and were repeated every hour. The last series of readings for the first day was taken eight hours after the beginning of the experiment. Each series of determinations comprised six to twelve separate readings. These measurements were repeated daily for at least four successive days and, whenever possible, they were continued over a period of several weeks. After the first day only one series of from six to ten readings was taken each day the experiment was conducted.

As has been described previously (2), the measurements made with the counter were corrected for background, decay of the radio-iodine, and non-linearity of the counter. The sensitivity of the counter was checked with a radium standard several times during each day the instrument was operated. For the six months' period during which these experiments were conducted, it varied less than 3 per cent. Background determinations also were taken at frequent intervals and were observed to vary but slightly. The value of 8.8 days for the half-life was used in correcting for the decay of the radio-iodine.[2] During the first day of each experiment the radiation from the rest of the body[3] made it difficult to determine accurately the radioactivity of the thyroid. However, since the radio-iodine was rapidly excreted (1), this problem became relatively unimportant after the first day.

[2] This value gives the half-life as measured by gamma ray radioactivity of the mixture of radioactive iodine isotopes used in these experiments.

[3] A description of the method of correction for radiation from the body is given in the appendix.

The radioactivity of the thyroid gland in situ was compared with an aliquot fraction of the radio-iodine given to the subject and the center of mass of the gland was estimated by palpation. From these data it was possible to calculate roughly the amount of radio-iodine taken up by the thyroid. Three of the patients underwent thyroidectomy and the radio-iodine content of the removed thyroids was determined by direct measurement using the method described in a previous report (1). A comparison of the values obtained by the measurement of the radioactivity of these glands in situ and after their removal revealed that in two instances the results agreed within 10 per cent. However, in the third case the estimated radioactivity of the thyroid in situ was 50 per cent lower than the direct measurement of the radio-iodine content of the extirpated thyroid. A possible explanation of this discrepancy was that at operation the left lobe of the gland was found to be larger than had been expected and to extend behind the trachea. No corrections were made for the absorption of gamma rays in the thyroid gland and overlying skin and muscle. The gamma rays of the eight-day radio-iodine were observed by Livingood and Seaborg to possess an energy of 0.4 MEV (3) and are therefore sufficiently penetrating to enter the counter tube without significant diminution after passing through the thyroid and the overlying tissues.

Results. The patients studied by this technic included five adults with thyrotoxicosis, two adults with non-toxic goiter and normal metabolic rate, four children with hypothyroidism and without goiter, and one child with a goiter and hypothyroidism. Five normal subjects served as controls. None of the normal subjects and adult patients had received any iodine for at least six months prior to these experiments. The five children with hypothyroidism had received small quantities of iodine in the form of dried thyroid substance until six weeks before the administration of the radio-iodine. The clinical diagnosis was established in each case by thorough medical examination and complete laboratory studies. The latter included one or more determinations of the basal metabolic rate in all instances and galactose tolerance tests in the thyrotoxic patients. The diagnosis of hypothyroidism in the five children was based upon findings of low basal metabolic rate, elevated blood cholesterol, retarded bone age, and delayed mental development. The five normal subjects had complete medical examinations within a year of these studies and determinations of the basal metabolism at the time of the experiments.

The curves shown in figures 1 and 2 represent the content of radio-iodine in the thyroid glands plotted against time. The abscissae indicate the time in days following the administration of the radio-iodine and the ordinates represent the percentage of uptake of radio-iodine. Because of the limitations of space, only the first five days of the experiments are shown in the figures.

The relative heights of the individual curves of the five normal subjects (fig. 1) show the apparent variations in amounts of radio-iodine taken up by their thyroid glands. These differences are reasonably accurate because the thyroids were roughly similar in size and shape as far as could be determined by careful palpation. The curves closely resembled each other in form although there was considerable variation in their maximum values. In each of the five experiments, the greatest uptake of radio-iodine by the thyroid apparently was not reached until

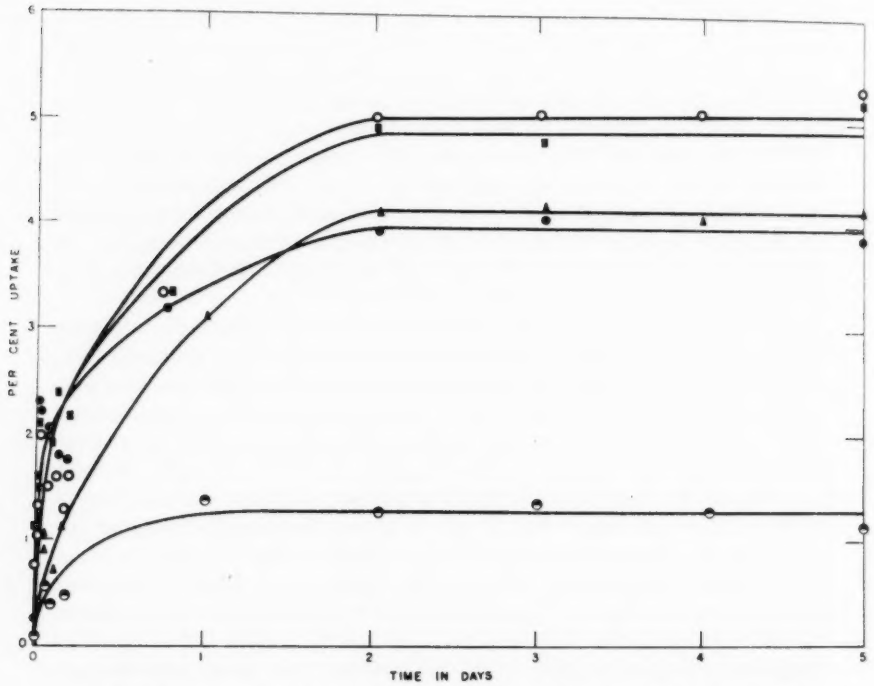

Fig. 1. Concentration of radio-iodine in the thyroids of five normal subjects.

two days following administration. The measurement of the radioactivity of the glands was continued for twenty-nine days in four subjects and for eight days in the fifth subject. After the second day of each of the five experiments, there was no further change in the form of the curves within the limits of experimental error. The estimated uptake of radio-iodine by the thyroid glands at the end of the second day ranged from 1 to 5 per cent with an average value of 3.5 per cent. The error in the measurement of radio-iodine uptake for each gland was probably less than 25 per cent.

In figure 2 are shown composite curves for the normal subjects, the hypothyroid children without goiter, the patients with non-toxic goiters, and the patients with hyperthyroidism. The single curve obtained from the child with a goiter and hypothyroidism is also included in this diagram.

The curves of radio-iodine uptake of the thyroids of the two patients with non-toxic goiters and normal basal metabolic rates were similar in form to those observed in the normal subjects. The only significant differ-

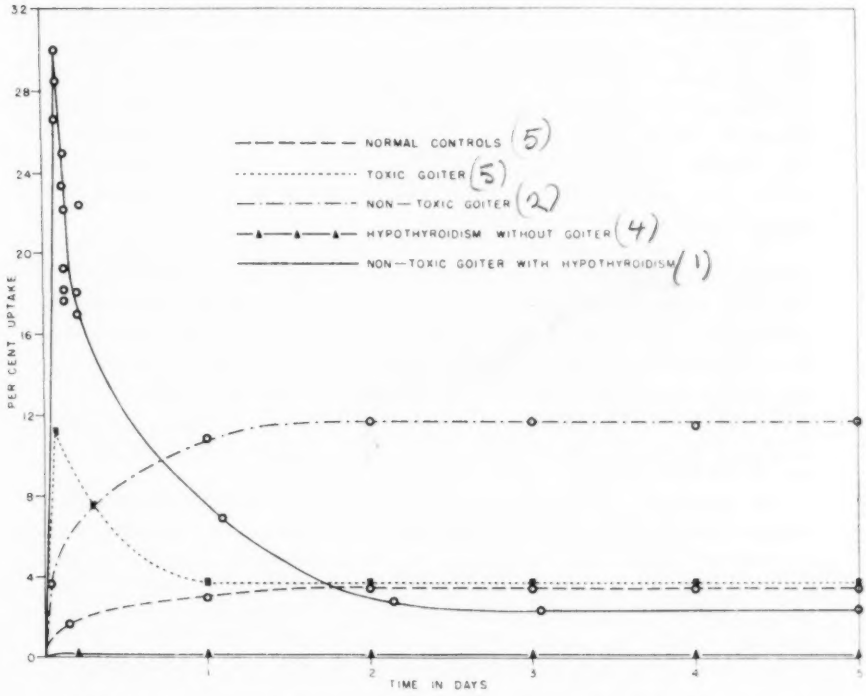

Fig. 2. Concentration of radio-iodine by the thyroids of 1, five normal subjects; 2, five adults with hyperthyroidism; 3, two adults with non-toxic goiters; 4, four children with hypothyroidism; and 5, one child with a goiter and hypothyroidism.

ence was that the percentage of radio-iodine concentrated in the thyroids of these two patients was much greater than in the normal group. The thyroids of these two patients showed no measurable change in radio-iodine content after the second day of the experiments. One patient was studied for six days and the other for two weeks. In one the uptake of radio-iodine in the thyroid at the end of the second day was 12 per cent of the administered dose, in the other it was 10 per cent.

The curves obtained from the five patients with thyrotoxicosis (fig. 2) differed strikingly in form from those of the normal group and the two adult patients with non-toxic goiter. The greatest concentration of radio-iodine in the thyroids of the thyrotoxic patients occurred at from one to four hours after its administration. Thereafter the radioactivity fell rapidly and at the end of twenty-four hours it was from one-half to one-fifth of its maximum value. The content of radio-iodine did not change significantly during the next four days. The study had to be terminated at the end of five days in two of the patients. The thyroid of the third patient lost approximately 25 per cent of the accumulated radio-iodine between the end of the second day and the tenth day, and the glands of the fourth and fifth patients showed no measurable changes in content of radio-iodine during the same period of time. The maximum uptake of radio-iodine in the thyroids of these five patients during the first day varied from 7.4 to 20 per cent. The amount of radio-iodine in the glands at the end of two days ranged from 1.5 to 7.1 per cent.

The uptake curve of the child with a goiter and hypothyroidism was similar to the curves observed in the thyrotoxic patients. The thyroid of this child attained its maximum uptake of 30 per cent of the administered radio-iodine at the end of one and one-fourth hours. Twenty-four hours later the content of radio-iodine fell to one-sixth of this value, and at the end of two days it had decreased an additional 50 per cent to 2.5 per cent of the administered amount. During the following three days it diminished approximately 10 per cent. The experiment was concluded at the end of the fifth day.

The uptake of radio-iodine by the thyroids of the four hypothyroid children without goiters ranged from 0.02 ± 0.01 per cent to 0.08 ± 0.03 per cent with an average value of 0.05 ± 0.02 per cent. These measurements are subject to considerable error because of the very small amounts of radio-iodine accumulated by the presumably atrophic thyroids of these patients.

Discussion. By the method of study we described, it was possible to follow the iodine metabolism of the thyroid gland in situ over a period of several weeks. Since the weight of iodine and the content of radio-iodine of each sample were determined before the solutions were administered and since the non-radioactive and the radioactive iodine remain in constant proportion in their passage through the body, the actual amount of iodine taken up by the thyroid gland could be calculated. For example, if a thyroid were found to contain 10 per cent of radio-iodine, then it also contained 10 per cent, or 1.4 mgm. of the 14 mgm., of the non-radioactive iodine which had been administered to the subject.

The advantages of following the uptake and discharge of iodine from the thyroid of a single subject over a period of many days are obvious.

The only other method of following iodine metabolism in various types of thyroid disorder would be to remove the thyroids in groups of subjects with thyroid diseases at varying intervals after the administration of iodine. This type of experiment is more time consuming and expensive; and, since it requires many more subjects, it is best applied to animal work.

The curves for the normal subjects and for the patients with goiter but without measurable thyroid dysfunction seem to indicate that the thyroids in both groups take up about the same amount of iodine per gram of thyroid tissue and that they retain this amount for periods of days to weeks. While the uptake curve was smooth and reached its maximum at the end of about 48 hours, the error of determination was such that fluctations within 0.3 per cent of the dose of administered iodine were obscured during the first 24 hours. On the other hand, the thyroids of the five patients with hyperthyroidism and of the one patient with a goiter and hypothyroidism took up iodine much more rapidly but were unable to retain it. It is, of course, impossible from our data to know the chemical linkage of the iodine discharged from the thyroids in the latter group.

It has been shown by pathological examination that the thyroids of patients with spontaneous myxedema are usually fibrosed and atrophic. If this were true also of the thyroids of the non-goitrous children with either severe hypothyroidism or with childhood myxedema, then the very small uptake of iodine (0.003 to 0.01 mgm.) was to be expected. Apparently the iodine concentrated in their glands was not sufficient to produce the amount of thyroid hormone required for the maintenance of a normal "thyroid" balance.

SUMMARY

The iodine metabolism of the thyroid glands of five normal subjects, five patients with hyperthyroidism, two patients with non-toxic goiters, four hypothyroid children without goiters and one patient with goiter and hypothyroidism has been studied by the use of radioactive isotopes of iodine. The uptake of iodine by the thyroids when measured over a period of five days or longer shows a characteristic curve for each of these groups. Some of the theoretical considerations which are based on the knowledge of iodine metabolism gained from this work and from the work of others have been discussed.

Appendix. In order to obtain the correct value for the radioactivity of the thyroid gland during the first two days of each experiment, a series of determinations of the radiation from the rest of the body was made by measuring the intensity of gamma radiation at the back of the neck just below the seventh cervical vertebra. The distances of this point and of the thyroid gland from the rest of the body were approximately the same. As the thickness of skin and of deeper tissues underneath the counter tube in these two positions was similar, the intensity of the gamma

radiation from the body at these two points was approximately the same. The gamma rays measured with the counter over the thyroid were the sum of the radiations from the thyroid and from the rest of the body. When the counter was against the back of the neck, the intensity of the gamma rays at this point was the sum of the gamma radiations from the body and of the small proportion of gamma rays which penetrate the neck from the thyroid.

The proportion of the gamma rays arising from the thyroid which passed through the neck was determined daily in each experiment by measuring the radioactivity in the two positions described. This proportion became constant at the end of the second day after the administration of the radio-iodine and did not change throughout the remainder of each experiment. This was due to the fact that the body radiation fell to a negligible value at the end of this time because of the rapid excretion of radio-iodine.

The quantity of radiation penetrating to the back of the neck from the thyroid gland varied from 8 to 10 per cent in the normal subjects and in the patients with small goiters. It ranged from 15 to 20 per cent in the patients with large goiters and in the one goitrous child included in this series.

The formula employed to obtain the correct value for the radioactivity of the thyroid glands during the first two days of each experiment is given below. After the second day no correction for the radiation from the rest of the body was necessary. The formula was derived in the following manner: where T_f is the measured radioactivity at the front of the neck over the thyroid, T_b is the measured radioactivity at the back of the neck, T_c is the corrected value for the radioactivity of the thyroid, R is the proportion of the radiations from the thyroid which penetrate to the back of the neck, and B represents the radioactivity arising from the rest of the body. Then:

(1). $T_f = T_c + B$ and (2). $T_b = RT_c + B$. Subtraction of (2) from (1) gives equation (3): $T_f - T_b = T_c - RT_c$. Solving for T_c, (4) $T_c = \dfrac{T_f - T_b}{1-R}$

The principal source of error in this method of computation lay in the fact that the assumed equivalence of body radiation at the thyroid and at the back of the neck was an approximation. Fortunately, during the first day of the experiments the measured intensity of radiation over the thyroid of each goitrous patient was from three to six times greater than it was over the back of the neck. This difference of intensity appeared within less than an hour after administration of the radio-iodine. Since the amount of radiation penetrating the back of the neck from the thyroid was observed to be from 8 to 20 per cent of the radioactivity of the gland, it was obvious that a large proportion of the measured radioactivity at the back of the neck arose from the thyroid. Therefore, even if the body radiation varied by as much as 50 per cent between the front and the back of the neck, the error would be relatively small for the corrected value of the radioactivity of the thyroid gland. The error in this group was estimated to be less than 10 per cent during the first day of the experiments.

The uptake of radio-iodine of the thyroid glands of the five normal subjects was considerably less during the same period of time. At the time of the last determination of the first day, the estimated quantity of radio-iodine taken up by the thyroid glands of two of this group was less than 0.7 per cent. In these two instances the error for the corrected value of the radioactivity of the thyroids was less than 50 per cent. The uptake of radio-iodine by the thyroids of the remaining three normal subjects varied from 2 to 2½ per cent during the same period of time, and the error was estimated to be less than 20 per cent.

Since most of the administered radio-iodine was excreted during the first day, it was to be expected that after the first day the error in accurate determination of the radioactivity of the thyroid glands due to radioactivity from the rest of the body would be small in both the patients and the normal subjects. Direct corroboration of this was observed in each experiment since the ratio of measured radioactivity at the back of the neck to that of the thyroid decreased, on an average, less than 5 per cent during the second day. Thereafter, as has been previously mentioned, this ratio remained constant within the limits of experimental error.

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THE EFFERENT PATHWAY FOR REFLEX PUPILLO-MOTOR ACTIVITY[1]

ROBERT HODES

From the Department of Physiology in the Harvard Medical School

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Like many autonomic effectors, the iris is supplied by both divisions of the enterofective nervous system. The double innervation and the existence of autonomic antagonism affords the opportunity to study whether reciprocity exists between the centers involved in the elicitation of reflex changes in pupillary dimensions—whether, for instance, reflex dilatation is brought about by a simultaneous decrease of oculomotor tone and increase of discharge over the sympathetics.

Since Vulpian (1878) established the fact that reflex dilatation may occur in the absence of the sympathetic innervation, it has been accepted that the reflex can be brought about by third nerve inhibition. The conflicting data in the voluminous literature concern the rôle of the sympathetic system in the process. One group of investigators, e.g., Budge (1855), Luchsinger (1880), Guillebeau and Luchsinger (1882), Steil (1894), Anderson (1903) and Keller (1932) considers the participation of the smypathetic in reflex dilatation an established fact. On the other hand, Bechterew (1883), Braunstein (1894), Lieben and Kahn (1930), Bain, Irving and McSwiney (1935), and Ury and Gellhorn (1939) indicate that the sympathetic is of minor importance or else plays no part in reflexly evoked dilatation.

The following experiments were undertaken in an attempt to control for some of the possible errors in previous investigations, and to provide additional data relative to theories of the rôle of the sympathetic system in regulating the caliber of the pupil in various physiological conditions.

METHODS. Cats were used exclusively. The operations performed were: 1, complete sympathectomy; 2, aseptic removal of the ciliary ganglion; 3, acute, and 4, chronic intracranial section of the oculomotor nerve; and 5, unilateral cervical sympathectomy.

Removal of the sympathico-adrenal system was accomplished in two or three stages, with aseptic precautions under ether anesthesia, according to

[1] While this paper was in press Seybold and Moore have published (J. Neurophysiol. **3**: 436, 1940) the conclusion that inhibition of the oculomotor nerve is responsible for pupillary dilatation of reflex and emotional origin.

the method of Cannon, Newton, Bright, Menkin and Moore (1929). In some animals one or both cervical sympathetic trunks were excised, or unilateral or bilateral superior cervical ganglionectomy was performed. In most instances experiments on sympathectomized animals were undertaken without the use of anesthetics and after the cats had recovered from the effects of the extensive surgical intervention. In some cases the sympathectomized animals were sacrificed in an acute final experiment under urethane anesthesia.

In one animal the ciliary ganglion on one side was excised according to the technique described by Shen and Cannon (1936). The cat recovered uneventfully and was studied at different times for 2 months thereafter. It was then used in a final experiment performed under urethane.

A series of cats was prepared in which one oculomotor nerve was cut within the skull in an acute experiment. The reactions of the pupils to light, sensory stimulation, asphyxia and varying degrees of anoxia were then studied. Acute third nerve destruction was performed under nembutal, dial, urethane, or ether. Ether, when used, was maintained only during the course of the operation; after severing the oculomotor nerve the effects of the volatile anesthetic were allowed to wear off before the experiment was begun. The method of destroying the third nerve consisted of exposing the brain-stem by removal of one or both cerebral hemispheres and gently lifting the brain-stem upwards, thus uncovering the nerve as it emerged from the midbrain. It was then cut by using a long-handled sharp curved hook. Successful section was indicated by a sudden dilatation of the pupil on the operated side, while the control pupil remained unaltered. Although the operation was at first time-consuming and attended by considerable hemorrhage, with a little practice the technique was improved so that the nerve could be cut with slight blood loss in 30 minutes or less. The animals were left on a warming pad and allowed to recover from the operation for about an hour or more before any experimental procedures were instituted.

Third nerve section in cats which were to be studied over a long period of time was conducted in essentially the same manner as in the acute preparations. The anesthetic, however, was always nembutal (0.6 to 0.7 cc. per kgm. intraperitoneally), and strict asepsis was of course observed. Also, in order to have an animal with as much of the central nervous system intact as was consistent with the demands of the operation, partial hemidecortication was elected. The motor areas in these animals were intact, as it was found feasible to destroy the oculomotor nerve by removing only the occipital lobe and parts of the parietal and temporal cortices. After the successful operations the animals recovered without complications; they ate well and had regained or exceeded their original body weight by the time the experiments were started. The observations on

these animals were eventually terminated by studying the actions of various experimental procedures under anesthesia.

In anesthetized animals, adrenaline effects were routinely abolished by tying off each adrenal gland *en masse*.

Pupillary changes were usually observed, in the unanesthetized animal, by simple inspection. Occasionally animals were placed in a box, the head was held by an assistant, and the pupils were photographed under constant illumination. In anesthetized animals the pupils were sometimes photographed or were measured by placing a pair of calipers directly on the cornea. The most usual method of measuring was as follows. Indelible ink lines, 1 mm. apart, were marked on small pieces of thin cellophane with a fine ruling pen. The lids were cut and retracted and the nictitating membranes (usually relaxed) were retracted or, in some cases, excised. The strips of cellophane, then placed on each cornea, gave a direct measure of the size of the pupils.

Stimuli of different sorts were employed to study their effects on the pupils. Flashing a bright light into the eye or taking the animal into a darkened room was used to study the reactions to varying light intensities. Emotional stimulation of unanesthetized cats was supplied by confronting them with barking dogs or by producing loud and sudden noises, etc. Handling or applying weak induction shocks to the skin provided disturbing stimulation in both chronic and acute experiments. When varying degrees of anoxia were required the tracheal cannula was connected with a Tissot spirometer which contained low-oxygen mixtures. Asphyxiation resulted from attaching a rubber bladder to the tracheal cannula, thus forcing the animal to rebreathe its expired air. The central stump of the bared sciatic nerve was used for afferent stimulation; shielded silver-wire electrodes were placed on the trunk and the stimulating current was derived either from a Harvard inductorium (faradic shocks) or from a multivibrator (condenser discharges of varying frequencies and intensities).

The drugs employed in these experiments were eserine salicylate (Sharp and Dohme), acetylcholine chloride (Merck), pilocarpine nitrate (Merck), and adrenalin chloride (Parke-Davis).

Results. 1. *Completely sympathectomized cats.* In such animals the pupil is small. That this constrictor tone may be suppressed has been abundantly confirmed since the early work of Vulpian (1878). That the adrenal glands are not required for the appearance of pupillary dilatation was demonstrated by Bain, Irvin and McSwiney (1935). The possible action of other humoral agents, however, has not been hitherto excluded. Sympathectomy offers a certain method of obviating sympathetic humoral effects, since the release of adrenaline or sympathin into the blood stream is no longer possible.

A few sympathectomized cats were prepared especially to study the

reactions of the iris, but most of the observations were made on cats which were used for other experiments and had been deprived of their sympathico-adrenal systems 2 weeks to 3 months previously. At least 25 such animals were examined. In all cases, struggle, excitement, or sensory stimulation, produced by a number of methods, was attended by prompt pupillary dilatation. The same result followed whether or not the cervical sympathetic trunks or superior cervical ganglia had been previously excised. No contraction of the nictitating membrane occurred with the same types of stimulation. When the sympathectomized animals were sacrificed in a final experiment under anesthesia, faradization of the central end of the bared sciatic nerve elicited rapid and marked dilatation of the pupil, but had no action on the nictitating membrane.

B. *Cat with unilateral excision of the ciliary ganglion.* As is well known, removal of the ciliary ganglion leads to a widely dilated pupil. The observation of Anderson (1903) and of Shen and Cannon (1936), that the light reflex is absent in the parasympathectomized iris, was readily confirmed. In addition, I thought that by inducing an even more dilated pupil than was already present—e.g., by the injection of adrenaline—central inhibition of the sympathetic might be unmasked. Even with such a wide pupil exhibition of an intense light elicited no constriction. The converse of this experiment also failed to point to sympathetic participation in the light reflex: both eyes were treated with pilocarpine; when maximal myosis had occurred the cat was placed in a dark room for 20 minutes. The pupil on the normal side dilated under these conditions, while the parasympathetically denervated iris was unaffected by the dim light, and the pupil remained quite narrow.

Emotion or afferent stimulation caused no widening on the side deprived of its oculomotor nerve, although the usual dilatation took place on the intact side. Even after the local instillation of myotic drugs had narrowed the pupils, stimuli of various sorts evoked no dilatation of the denervated side, although they caused marked and prompt expansion of the pupil on the normal side. The drugs employed did not alter the reactivity of the pupil, since direct stimulation of the sympathetic trunk in the neck elicited rapid dilatation.

It is interesting to note that in some animals *both* nictitating membranes were withdrawn on afferent stimulation, while the same stimulation caused retraction of the iris only when the third nerve was intact. This is in agreement with an observation of Karplus and Kreidl (1918). On the other hand, Keller (1932) concludes that manipulation of the midbrain animal causes increased sympathetic activity (retraction of the nictitating membrane, separation of the lids and dilatation of the pupil) since these reactions are not present on the sympathectomized side. But in the protocols of his cats 225 and 226 I find no indication that the *irises* on the two

sides behaved differently, although there is an undoubted difference between the activity of the membranes and eyelids on the two sides. This absence of parallelism between pupillary and other ocular effects should point out the obvious fallacy of inferring a sympathetic effect on the iris simply because it has such an action on the nictitating membrane.

The following excerpts from the protocol of this cat indicate the salient features of the results.

Protocol of cat 1—♂, 3.4 kgm.

11/22/38. Left ciliary ganglion excised aseptically under ether anesthesia.

11/24/38. Left pupil wide and insensitive to light. Right pupil normal.

12/ 5/38. No reflex to bright light on left side has been obtained since operation.

12/ 6/38. 0.5 mgm. adrenaline injected subcutaneously. Left pupil widened slightly, right dilated markedly. Bright light flashed into eyes. Right pupil constricted rapidly, left unaffected.

1/ 3/39. 2:25 p.m.—1 drop 0.4 per cent pilocarpine nitrate instilled in each conjunctival sac.
2:40 p.m.—Both pupils constricted to slits.
2:40–3:00—Cat in dark room. Right pupil wider than before being taken into room; left remained the same as at 2:40.

1/ 5/39. 9:38 a.m.—2 drops 1 per cent eserine salicylate in each eye.
9:50 a.m.—Pupils narrow. Left smaller than right.
10:05 a.m.—Dog brought into room. Cat excited. Right pupil widened when dog was noticed, left pupil unaffected.

1/22/39. Acute experiment. Urethane. Adrenals tied. Cellophane markers on each cornea.
12:25 p.m.—Left pupil 14.0 mm., right 3.5 mm. Both nictitating membranes relaxed.
12:47 p.m.—2 drops 1 per cent eserine instilled in each eye.
12:59 p.m.—3 drops 0.1 per cent acetylcholine put in each conjunctival sac.
1:38 p.m.—Left pupil 2.3 mm., right 3.0 mm.
1:40 p.m.—Stimulation of central end of left sciatic nerve (1.5 volts, secondary at 8.5 cm.). No dilatation of left pupil; marked expansion on right side. Contraction of *both* nictitating membranes.
2:00 p.m.—10 γ adrenaline injected intravenously. Dilatation of both pupils and retraction of both nictitating membranes.
2:20 p.m.—Stimulation of cephalic end of cut left cervical sympathetic trunk (1.5 volts, 12 cm.). Sudden widening of pupil and contraction of membrane.

3. *Cats with acute third nerve section.* Ten cats were studied about an hour after the oculomotor nerve had been cut within the skull. The adrenal glands were routinely tied off; in most experiments the cervical sympathetic trunk was severed on the side opposite to the third nerve destruction.

The widely dilated pupil is insensitive to light. The loss of the light reflex is due solely to absence of the oculomotor nerve and not to other effects of the operation, e.g., removal of the cortex "shock," etc., since in

animals deprived of both hemispheres the iris with oculomotor nerve intact reacts normally to variations in the light intensity.

Afferent impulses—whether set up by direct electrical stimulation of the bared sciatic nerve, by pinching, pricking or faradizing the skin with induction shocks—never produced pupillary dilatation on the parasympathectomized side. Constriction of the wide pupil was frequently brought about by the use of myotic drugs, applied locally, but this procedure also failed to disclose dilatation of the pupil on afferent stimulation. "Shock" cannot explain these negative findings; for on the side with the third nerve supply intact the pupil dilated readily under the same conditions. Also, when the sympathetic was cut on the side opposite that on which the third nerve had been removed, stimulation of the sciatic caused the pupil on the sympathectomized side to dilate, while the opposite pupil remained fixed.

In 3 animals, in which the third nerve on one side was cut and both sympathetics were intact, sciatic stimulation elicited dilatation of only the normal pupil but retraction of *both* nictitating membranes (see p. 147).

Although the parasympathectomized iris could not be reflexly excited to produce expansion of the pupil, injection of adrenaline occasioned a further dilatation of the wide pupil. Also, direct stimulation of the cervical sympathetic trunk with weak induction shocks caused widening whether or not a myotic drug had been previously administered.

The following abbreviated protocol of cat 2 will serve to indicate the reactions of the pupils of a cat subjected to destruction of the oculomotor nerve on one side.

Protocol of cat 2—♂, 2.9 kgm.—3/9/39.
Urethane, adrenals tied. Right sciatic exposed and prepared for central stimulation. Cellophane marker on each cornea.

2:55 p.m.—Decortication and section of left third nerve completed.

4:07 p.m.—Right sciatic stimulated centrally (1.5 volts, 6 cm.). Rapid widening of right pupil; left pupil (11.0 mm.) unchanged. Retraction of both nictitating membranes.

4:12 p.m.—5 drops 0.1 per cent eserine in each conjunctival sac.

4:20 p.m.—2 drops 0.04 per cent acetylcholine in each eye.

4:52 p.m.—Left pupil, 3.5 mm., right, 4.0 mm.

4:58 p.m.—Stimulation of right sciatic. Right pupil 6.5 mm.; left remained at 3.5 mm.

5:12 p.m.—Stimulation of cephalic end of cut left cervical sympathetic nerve (1.5 volts, 12 cm.) produced rapid and wide dilatation.

The effects of low-oxygen tension and asphyxia were studied on some animals with one third nerve removed acutely. The results were not clear, since usually no change was observed until the animals were moribund. Generally, 5 to 7 per cent oxygen mixtures or asphyxiation caused, after 2 to 6 minutes, dilatation of both the normal and denervated sides. Dilatation occurred during oxygen lack also when the sympathetic had been

severed previously. One adrenalectomized animal, lacking the oculomotor nerve on the left and the cervical sympathetic on the right side, was asphyxiated almost to death. Both pupils dilated, the right widening more obviously from its resting position than the left. Asphyxiation was discontinued, and the animal recovered. Then the left sympathetic trunk was cut in the neck, thus leaving a completely denervated iris on this side. Asphyxiation was begun again and once more elicited widening of both pupils. The dilatation occurred slowly and synchronously on both sides. This experiment indicates that dilatation in this case was not of neural origin. It might be referred to some direct effects on the iris, due to asphyxia.

4. *Cats with chronic oculomotor nerve section.* In order to rule out beyond doubt the interference of "shock" and also to be able to dispense with anesthetics, intracranial third nerve section was performed in 3 cats which were then allowed to recover for at least 2 weeks before being used again. These animals exhibited pupils which were unresponsive to light, whether or not pupillo-constrictor or dilator drugs had been employed. In none of these animals did stimulation of the skin or excitement cause dilatation on the parasympathectomized side. Likewise, in the final experiment under urethane, sciatic stimulation failed to indicate any participation of the sympathetic centers in the process of reflex dilatation.

A protocol of one experiment on cat 3 exemplifies the typical data obtained in several observations on the 3 animals.

Protocol of cat 3—♀, *2.2 kgm.*

6/ 2/39. Right third nerve cut within skull under nembutal. Motor areas intact.

6/ 5/39. Cat well; eating of own accord. Right pupil wide and fixed to light. Left side has normal reactions to light.

6/31/39. Cat has recovered from operation and is eating well. Right pupil wide and insensitive to bright or dim light.

6/22/39. 11:07 a.m.—One drop 1 per cent eserine in each conjunctival sac.
11:30 a.m.—One drop 1 per cent acetylcholine in each eye.
11:40 a.m.—Both pupils narrow; left slightly larger than right.
12:00—Cat put in box and became excited. Left pupil widened rapidly; right unaffected. Other stimuli—pinching the pinna, pricking the skin, pulling whiskers, stimulating the skin with weak induction shocks, and confronting it with a barking dog—also caused the left pupil to dilate, but the right side remained unaltered.

5. *Cats subjected to unilateral cervical sympathectomy.* A final series of experiments was devised to test for a possible participation of the sympathetic outflow in reflex pupillary dilatation. An attempt was made, after tying off the adrenal glands and cutting one sympathetic trunk in the neck, to find a stimulus which would induce reflex expansion of the pupil on the normal side without affecting the denervated side. To this end varying strengths and frequencies of condenser shocks were applied to the bared

sciatic nerve and both eyes were observed for a possible differential effect of the stimulation. In addition, the animals were subjected to varying degrees of asphyxia in order to determine whether any differences would appear on the two sides.

All the foregoing experiments were negative so far as the activation of sympathetic centers in pupillary dilatation is concerned. Thus, if a series of shocks delivered to the sciatic nerve brought about an increase in the caliber of the normal pupil, dilatation also occurred on the denervated side. The dilatation began at the same time on both sides and both pupils ran a parallel course during and after stimulation. If the frequency of the shocks was too slow, no effect was seen on *either* side. When a stimulus was of insufficient intensity to cause a response on the denervated side, it also failed to elicit any change in the intact eye. As the strength of current was gradually increased, a point was reached at which both pupils dilated. No stimulus ever caused one pupil to widen without producing a corresponding effect on the other. Hence, no differential action could be observed by varying either the frequency or strength of the shocks applied to the sciatic nerve.

Differing degrees of asphyxia likewise failed to cause dilatation of one pupil without similarly affecting the opposite side. Deepening the narcosis did not alter either the effects of nerve excitation or asphyxia. The pupils in all cases began to dilate at the same time, remained wide concurrently, and constricted simultaneously when the stimulus was removed.

The sympathectomized side invariably presented a pupil which was narrower than the normal. Also, when the stimulus was acting, the pupil on the denervated side dilated less markedly than the control. This fact does not require the postulation of an increase of sympathetic discharge. It may be explained on the basis of an alteration of the normal sympathetic-parasympathetic balance. If a constant tonic activity by way of the cervical sympathetics is assumed, then a decrease of constrictor activity would lead to dilatation by two means: 1, a direct effect produced by the relaxation of the *sphincter pupillae*; and 2, a relative release of the dilator muscle from the balancing force of the opposed constrictor muscle. Since the second method is abolished when the sympathetic is destroyed, it is probable that the resultant widening of the pupil will be less extreme than when both mechanisms are utilized.

The following protocols are typical of 6 similar experiences which indicate that the sympathetic system plays no significant rôle in dilatation under the conditions investigated.

Protocol of cat 4—♀, *2.2 kgm.—3/23/39.*

Urethane. Adrenals tied. Right cervical sympathetic cut. Left sciatic prepared for central excitation. Cellophane marker on each cornea.

4:15 p.m.—Right pupil, 0.3 mm.; left, 1.3 mm. 4 minutes of stimulation of central

sciatic by condenser shocks (1 per sec., 2 volts) caused no change in either pupil.

4:21 p.m.—Right pupil, 0.3 mm.; left, 1.3 mm. After 2 minutes of stimulation (1 per sec., 4 volts) the right pupil was 1.0 mm., the left 2.6 mm. Both pupils began to dilate at the same time, reached the maximum width together, and returned to their original sizes together when the stimulation was halted.

4:37 p.m.—Right pupil, 0.3 mm., left, 1.0 mm; 2.5 minutes of stimulation (1 per sec., 1 volt) caused no change on either side. After 1 minute of stimulation at 4 per sec. and 1 volt, the right pupil was 1.0 mm., the left, 3.0 mm.

5:19 p.m.—Right pupil a slit; left, 1.5 mm. Stimulation at 15 per sec., 2 volts. After 30 sec. right was 2.0 mm., left was 3.5 mm. After 2 minutes of continuous stimulation, right pupil was 0.9 mm., left was 2.7 mm.

5:30 p.m.—Anesthesia deepened by injecting more urethane.

5:30-6:20—Series of intensity and frequency variations employed as before. Both pupils change together or not at all.

6:40 p.m.—Right pupil, 0.7 mm.; left, 3.0 mm. Tetanic stimulation, 4 volts. After 30 sec., right was 2.0 mm., left, 5.0 mm. After 1 minute of continuous excitation, right was 1.0 mm., left, 4.0 mm. After 2 minutes, right was 1.0 mm., left, 4.0 mm.

Protocol of cat 5—♂, 3.4 kgm.—5/16/39.

Dial. Adrenals tied. Right cervical sympathetic cut. Cellophane marker on each cornea.

4:20 p.m.—Right pupil, 1.5 mm., left, 3.0 mm. Empty rubber bladder attached to tracheal cannula. Both pupils began to dilate 35 sec. after asphyxiation was started. After 1 minute, right pupil was 2.0 mm., left, 6.0 mm. After bag was removed both pupils narrowed rapidly to original caliber.

Discussion. The material presented above points to the conclusion that reflex pupillary motor effects are mediated solely by the oculomotor nerve. Anderson's (1903) unsuccessful attempt to show a sympathetic component in the light reflex has been confirmed (p. 148). Gullberg, Olmsted and Wagman (1938), on the contrary, concluded that, in the rabbit, the sympathetic took part in the dilatation of dark-adaptation, since the widening was greater and perhaps more rapid on the normal than on the sympathectomized side. As has been stated (p. 151), this fact does not demand the positing of an increased sympathetic activity for its explanation. Gullberg, Olmsted and Wagman interpret the break in the curve (see their fig. 3) as being caused by the sympathetic acting by itself. Even if this assumption is valid, from their figure it is apparent that the change in the components causing dilatation occurs at approximately 100 seconds and not at 20 seconds as these authors state.

That constriction of the pupil is accomplished by the third nerve alone is accepted by all writers. Six main avenues of investigation have led to the hypothesis that when the pupil dilates in various physiological con-

ditions (emotion, asphyxia, afferent stimulation, etc.) the effect is mediated by increased sympathetic activity: 1, the demonstration by Bernard, Budge, and others that section of the cervical sympathetic resulted in pupillary constriction; 2, the work of Bernard (1852) which showed that direct stimulation of this nerve caused expansion of the pupil; 3, the researches of Karplus and Kreidl (1909) in which direct stimulation of the diencephalon evoked dilatation only if the sympathetic was intact; 4, Budge's (1855) stimulation of the dorsal roots of the isolated cord which elicited widening of the pupil; 5, the experiments of Luchsinger (1880) and Guillebeau and Luchsinger (1882) who, after transecting the cord below the medulla and cutting the sympathetic on one side, found that afferent stimulation brought about dilatation on the normal, but not on the sympathectomized side; and 6, the studies of Anderson (1903) which indicated that pupillary dilatation occurred during afferent stimulation even after the third nerve was destroyed.

The data presented in this paper are incompatible with the concept that sympathetic nervous mechanisms play any rôle in the production of reflex changes in the size of the pupil. The reasons why the above authors have reached an opposite interpretation of the part of the sympathetic may now be considered.

The studies mentioned in (1) and (2) above need not be considered, for they did not deal with *reflex* alterations. Also, the work of Karplus and Kreidl in no way forces one to accept reflex sympathetic pupillary effects, for descending fiber tracts from the diencephalon, which are not activated in dilatation of reflex origin, may have been stimulated.

When Budge (1855) isolated the section of the cord sending fibers to the iris from the rest of the cerebrospinal axis and found that stimulation of the dorsal roots led to prompt widening of the pupil, he considered this to be proof that the ciliospinal center had been reflexly aroused to action. An escape of current to the nearby ventral roots might have occurred to invalidate Budge's conclusions.

Luchsinger's (1880) statement that dilatation of the pupil resulted from afferent stimulation if the cord was cut above the ciliospinal center (but not after cervical sympathectomy in the spinal animal) has been refuted by Braunstein (1894) and by Bain, Irving and McSwiney (1935). Luchsinger employed strychnine, cocaine and other drugs, and it is possible that the discrepancy was due to the use of these pharmacological agents.

Anderson's (1903) report that sciatic stimulation or handling of the animal causes dilatation in the absence of the third cranial nerve cannot be accepted, for evidence against it has been reported by Bain, Irving and McSwiney (1935), Ury and Gellhorn (1939), and it is contradicted by the present experiments. Ury and Gellhorn suggested that Anderson utilized deep chloroform anesthesia and thus "functionally eliminated all higher

centers and created (by disinhibition) a state of heightened excitability of the ciliospinal center." Since Anderson mentions some dilatations of slow onset, it is also possible that humoral agents (adrenaline, sympathin) were not excluded.

Significant experimental data which have accumulated in the last decade—and which support the careful studies of Bechterew (1883) and particularly of Braunstein (1894)—make it increasingly apparent that reflex pupillary dilatation depends largely, if not completely, on depression of oculomotor tone. Lieben and Kahn (1930), on the basis of ablation experiments, attributed the mydriasis of emotional states to third nerve inhibition alone. Bain, Irving and McSwiney studied the effects of central splanchnic excitation on the size of the pupil; they eliminated the adrenal glands and also showed that large blood-pressure changes were not responsible for the ocular effects observed. Section of the third nerve or transection of the cord in the cervical region rendered the irises unresponsive to stimulation of the splanchnic nerves. Ury and Gellhorn urged that third nerve inhibition be accepted as the mechanism responsible for dilatation due to pain. They stated that only when the excitability of the ciliospinal center is raised (e.g., by metrazol) does the sympathetic add any component to the dilatation.

The present data add further support to the evidence of recent investigators who deny the contribution of the sympathetic in the process. This statement does not deny the importance of sympathetic humoral agents; indeed, the sympathetic humoral and the parasympathetic neural mechanisms coöperate to cause pupillary dilatation.

SUMMARY

1. Completely sympathectomized cats exhibit a normal light reflex. Physiological stimuli evoke pupillary dilatation which is indistinguishable from that of the normal animal (p. 146).

2. Unilateral excision of the ciliary ganglion, or acute or chronic destruction of the oculomotor nerve abolishes the light reflex. These operations remove the ability of the pupil to dilate to emotional, painful, or afferent nerve stimulation (pp. 147, 148 and 150; protocols of cats 1, 2 and 3).

3. Anoxia or asphyxia may cause dilatation on the parasympathectomized side, but this effect is considered to be due to other than sympathetic nervous mechanisms (p. 150).

4. No differences were established between a normal and sympathectomized eye by the use of a variety of frequencies and intensities of electrical stimulation of the sciatic nerve, or by asphyxiation of different degrees of severity (p. 151 and protocols of cats 4 and 5).

5. It has been concluded that, in the cat, under the conditions investi-

gated, no significant part in pupillary dilatation of reflex origin is played by the sympathetic nervous mechanisms.

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THE RELATION BETWEEN BIREFRINGENCE AND CONTRACTILE POWER OF NORMAL, HYPERTROPHIED, AND ATROPHIED SKELETAL MUSCLE

ERNST FISCHER

From the Department of Physiology and Pharmacology, Medical College of Virginia, Richmond

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All modern theories of muscular contraction take in account the submicroscopic crystalline arrangement of the muscle proteins as revealed by x-ray spectrography or birefringence observations (Fischer, 1936, 1940). If the crystalline structure really does play an important part in the contractile process, there should exist a certain parallelism between contractile power and the degree of completeness of the crystalline arrangement. The birefringence of a muscle is dependent to a large extent on this completeness of the crystalline pattern. The few birefringence data available in the literature for muscle of various species cannot be evaluated for this purpose. The contractile forces of these various muscles have not yet been determined accurately, and, what is much more important, birefringence analysis has revealed that the submicroscopical patterns of muscles of various species are not identical enough to permit the use of the data on total birefringence as a measure of the completeness of their crystalline arrangements (Fischer, 1940).

In an attempt to secure figures permitting such a comparison, the maximal isometric forces and the total birefringence of gastrocnemius-soleus muscles of rats were measured. Since the natural variations of the contractile power (tension per gram weight) and the birefringence of gastrocnemii from different rats are rather small, such a comparison is only possible on a very capacious experimental material; and even then the relations between strength and birefringence can be established only for a very narrow range. The cumbersome method needed for the determination of the average birefringence value of one muscle limits furthermore such a statistical investigation. Therefore, in this paper the main emphasis is laid upon the comparison of matching muscle pairs, one of them being normal, while the contractile power of the other has been either increased by previous training or decreased in consequence of denervation atrophy.

MATERIAL AND METHOD. All experiments are performed on rats 4 to 8 months old. The first series was on normal rats to find out the normal range of variations between matching gastrocnemii. In the second series, one hind leg of the rats had been stimulated twice daily for about twenty

minutes by faradic currents to produce a hypertrophy of the muscles of that side. By a motor-driven mechanical device the currents of an induction coil were turned on and off each half second, thus producing 60 short but strong contractions per minute. After one to two minutes of stimulation a rest period of about the same duration was interpolated and the direction of the currents changed. Thus each training period of 20 minutes included about 10 to 11 minutes of actual stimulation. Special care was taken to use just maximal stimuli. The animals were fastened to a rat-board and were kept under very light ether narcosis during actual stimulation. In the third series of experiments, one sciatic nerve had been resected aseptically for a length of 1 cm. at least several days or weeks previous to the final experiment.

Tension measurement. Under light ether anesthesia, both knee joints were fixed rigidly by clamps, the Achilles tendons exposed, then cut, and connected with a twin pair of isometric tension wire levers. The muscles were stimulated directly with repeated electrical shocks, optimal for strength, duration and frequency (Fischer, 1939), and the maximal tetanic tension was recorded on a smoked drum. After the tension measurement, anesthesia was interrupted and the rat released from its fixation. At least two hours were allowed for restoration of more or less normal blood supply in the stimulated muscles before the rat was killed by a blow on the head.

Weight and dry substance determination. Theoretically it would be correct to relate the total strength of a muscle to its physiological cross section (Fischer and Steinhausen, 1925). However, a determination of the physiological cross section of the gastrocnemius-soleus of the rat is rather impractical. Therefore, the tension per weight unit was chosen as a measure of the contractile power. Both muscles were dissected carefully and cleaned of other tissues, and the tendons were cut in exactly corresponding places. The muscles were weighed immediately and three to four muscle fiber bundles were cut out for the birefringence determination. Then the muscles were weighed again and dried at 108°C. to a constant weight.

Birefringence determination. The three to four small bundles of muscle fibers taken from each gastrocnemius (from corresponding parts of the matching muscles) were put in watch glasses with oxygenated buffered Tyrode solution. The bundles were then carefully teased into thinner bundles consisting of two or three fibers. The double refraction of these small fiber bundles was determined as phase differences under the polarization microscope by means of a graduated quartz wedge. The thickness of the bundles was measured by the use of the micrometer screw of the microscope. The latter measurement was facilitated by added traces of India ink to the Tyrode solution. The phase difference observed

was divided by the measured thickness of the bundles in order to find the birefringence per millimeter thickness. From the various values obtained for the 18 to 22 fiber bundles of a single muscle, the average value for the birefringence of that muscle was computed. The birefringence mentioned in this paper refers always to what is known as "total birefringence" since no attempt has been made to analyze the measured birefringence concerning its two components: "crystalline birefringence" and "form birefringence."

RESULTS. The first series, that on normal muscles (table 1), was used mainly to establish the normal variations, which must be expected between left and right gastrocnemii. The maximal variation in wet or dry weight

TABLE 1

Comparison of left normal with right normal gastrocnemius in rats

NO.	WET WEIGHT		DRY WEIGHT		TENSION		GRAM TENSION PER GRAM WET WEIGHT	GRAM TENSION PER GRAM DRY WEIGHT	TOTAL BIREFRINGENCE	
	Right	Left	Right	Left	Right	Left	Left in % of right		Right 10^{-4}	Left in % of right
	grams	*grams*	*gram*	*gram*	*grams*	*grams*				
1	2.049	2.067	0.468	0.468	1700	1750	102.0	102.2	2.38	101.3
2	1.768	1.732	0.393	0.395	1950	1950	103.7	99.6	2.59	99.6
3	2.017	2.038	0.473	0.478	1450	1500	102.1	102.3	2.29	97.6
4	2.266	2.331	0.548	0.557	1800	1850	99.7	101.3	2.33	100.9
5	1.952	1.927	0.457	0.452	1700	1650	98.5	98.4	2.27	100.0
6	2.080	2.102	0.494	0.492	1950	1950	99.1	100.7	2.38	99.3
7	2.712	2.749	0.623	0.647	2350	2450	103.1	100.4	2.34	98.6
8	2.271	2.285	0.542	0.549	2150	2050	94.8	94.2	2.26	101.0
9	2.141	2.171	0.509	0.514	1900	1950	101.4	101.1	2.35	101.4
10	2.100	2.085	0.502	0.496	1850	1850	100.8	101.2	2.40	99.6

observed for matching normal muscles is 2.9 per cent. The maximal difference for tension per gram weight reaches 5.2 per cent for wet weight and 5.8 per cent for dry weight. The maximal birefringence difference is 2.4 per cent. The average variations are much less, and table 1 indicates clearly that contractile power as well as birefringence of matching muscles can be regarded as practically equal.

In the second series, that with muscle hypertrophy produced by electrical training, some of the rats responded poorly to the training. However, for the problem in which we are interested in this paper, it is only of importance that hypertrophy could be produced at least in a large number of rats. For the sake of clearness table 2 includes only those animals in which the increase in weight of the trained muscle was at least twice as large as the maximal weight variation observed between pairs of normal

muscles. The observed tension increase of the trained muscles corresponds to their weight increase. In consequence the values of tension per gram weight are practically unaltered by the training. Also the birefringence of the trained muscles is the same as that of their untrained partners.

In the third series, comparing atrophying muscles with their normal partners (table 3), somewhat similar results were obtained as long as the atrophy did not become too severe. No appreciable diminution in birefringence or in tension per gram weight (the latter showing even the tendency to increase) occurs during the first 9 to 11 days, although the weight loss and loss in total tension proceed to about 30 per cent at the end of that

TABLE 2

Comparison of right trained with left normal gastrocnemius in rats

DAYS IN TRAINING	WET WEIGHT		DRY WEIGHT		TENSION		GRAM TENSION PER GRAM WET WEIGHT	GRAM TENSION PER GRAM DRY WEIGHT	TOTAL BIREFRINGENCE	
	Left	Right	Left	Right	Left	Right	Right in % of left		Left 10^{-3}	Right in % of left
	grams	*grams*	*gram*	*gram*	*grams*	*grams*				
5	1.560	1.670	0.374	0.415	1550	1650	99.4	96.2	2.53	101.4
10	2.045	2.240	0.488	0.528	2350	2550	99.1	100.3	2.60	98.5
12	1.617	1.883	0.399	0.465	1800	2150	102.4	102.7	2.47	99.5
13	1.529	1.692	0.365	0.407	1450	1600	99.4	98.8	2.35	98.0
14	2.059	2.250	0.443	0.486	1600	1750	99.9	99.6	2.28	99.1
16	2.376	2.606	0.577	0.625	2450	2750	102.1	103.6	2.57	98.8
17	2.015	2.277	0.472	0.536	1750	2000	101.2	100.8	2.22	99.1
18	1.754	1.939	0.417	0.469	1450	1600	99.8	98.1	2.26	100.9
18	1.541	1.775	0.362	0.423	1650	1900	100.2	98.7	2.43	98.3
25	1.806	2.048	0.436	0.494	1700	1950	101.1	100.0	2.38	101.4
41	2.010	2.349	0.450	0.523	1950	2300	101.0	101.4	2.50	100.9
47	1.971	2.293	0.445	0.517	1750	2100	103.4	103.4	2.45	98.9
54	1.785	2.187	0.443	0.537	1700	2100	100.2	101.8	2.50	99.2

period. However, in the later phase of atrophy, both tension per gram weight and birefringence drop considerably, but approximately at the same rate. Thus, for the second stage of atrophy, the loss in completeness of crystalline structure of the muscle substances corresponds roughly to the loss in contractile power.

Figure 1 represents graphically the absolute values for birefringence and tension per gram fresh weight of all normal muscles of the three series (dots), of the trained muscles (crosses), and of the denervated muscles (circles). Since training the muscles neither changed their birefringence nor their specific strength (table 2), the values for these muscles can be regarded also as values for normal muscles. It is obvious from the graph

that for normal muscles there exists statistically a relation between birefringence value and contractile power, the latter increasing with an increase of the former. Since the number of observations is limited and the range of natural variations is small, this relation between birefringence and

TABLE 3

Comparison of left atrophied with right normal gastrocnemius in rats

DAYS OF ATROPHY	WET WEIGHT		DRY WEIGHT		TENSION		GRAM TENSION PER GRAM WET WEIGHT	GRAM TENSION PER GRAM DRY WEIGHT	TOTAL BIREFRINGENCE	
	Right	Left	Right	Left	Right	Left	Left in % of right		Right 10^{-3}	Left in % of right
	grams	*grams*	*gram*	*gram*	*grams*	*grams*				
3	2.226	1.973	0.511	0.454	2050	1850	102.0	101.6	2.45	98.4
5	2.524	2.218	0.610	0.523	1950	1700	99.6	101.7	2.23	99.6
6	1.815	1.522	0.428	0.359	1800	1500	99.4	99.3	2.42	101.2
7	2.091	1.689	0.494	0.395	2150	1800	103.8	104.7	2.49	99.1
8	1.877	1.421	0.456	0.334	1750	1300	98.1	101.8	2.50	99.5
9	1.861	1.432	0.450	0.327	1850	1450	101.9	107.9	2.45	101.2
9	2.929	2.060	0.673	0.464	2350	1800	109.1	111.1	2.26	101.8
10	1.971	1.109	0.444	0.242	2350	1350	104.2	105.1	2.61	97.6
10	1.619	1.161	0.397	0.270	1950	1300	92.6	98.2	2.54	96.9
11	2.381	1.615	0.561	0.363	2450	1700	102.7	107.2	2.48	97.5
11	2.085	1.308	0.495	0.295	2150	1300	96.5	101.3	2.44	93.6
11	1.804	1.256	0.422	0.276	1850	1350	104.9	106.1	2.36	98.7
11	2.455	1.635	0.589	0.360	2550	1700	100.0	109.1	2.46	98.4
12	1.438	0.550	0.331	0.130	1450	450	81.4	78.9	2.48	80.6
12	2.650	1.703	0.622	0.382	2350	1450	96.1	100.5	2.38	94.0
13	2.474	1.380	0.575	0.309	2250	1050	83.7	86.9	2.32	83.7
15	2.287	1.204	0.525	0.275	2150	850	75.0	75.6	2.44	75.9
16	1.543	0.590	0.364	0.131	1450	450	80.3	86.2	2.37	82.4
17	1.985	0.893	0.464	0.193	1950	700	80.0	85.7	2.30	78.7
19	1.537	0.832	0.362	0.182	1500	650	80.0	86.1	2.54	74.1
20	1.854	0.842	0.437	0.194	1850	500	59.5	60.9	2.44	67.2
21	1.744	0.626	0.389	0.132	2100	600	79.5	84.0	2.58	71.4
22	1.609	0.595	0.375	0.136	1950	600	82.9	84.7	2.63	65.4
23	2.056	0.804	0.484	0.175	2100	550	67.0	72.7	2.43	71.3
26	1.226	0.529	0.278	0.113	1200	350	67.8	71.6	2.45	56.1
29	1.906	0.621	0.464	0.141	2150	500	70.5	80.0	2.47	67.0
33	1.793	0.561	0.416	0.117	1850	350	59.9	67.4	2.34	63.3

tension per gram weight could be represented by a straight line as well as by the curve drawn in figure 1. The latter is a logarithmic curve and corresponds to the function: birefringence = k × log. contractile power. The values for atrophying muscles do not correspond to this equation. For the muscles in the first stage of atrophy, the values fall in the range for

normal muscles. However, during the second stage of atrophy, the loss in contractile power is less than one ought expect according to the relation between birefringence and contractile strength in normal muscles. In the second stage of atrophy, birefringence changes more or less proportionally to the contractile power, and not to the latter's logarithm.

That in the first stage of atrophy, despite a weight loss up to 30 per cent, no change in birefringence nor in contractile power occurs, indicates that the weight loss during atrophy is a more or less independent process, not directly connected with the possible degenerative changes resulting in destruction of the submicroscopical structure and diminution of con-

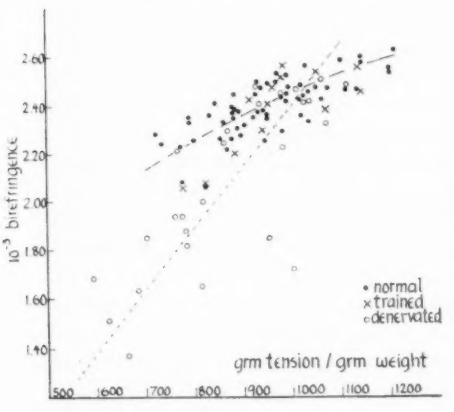

Fig. 1

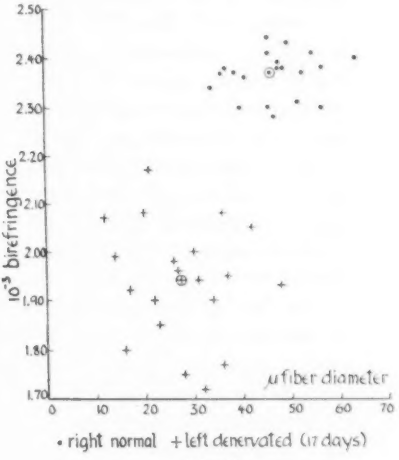

Fig. 2

Fig. 1. Relation between birefringence and contractile power (gram tension per gram wet weight) for normal, hypertrophied and atrophied muscles.

Fig. 2. Birefringence values and diameters of 20 individual fibers of the normal and the atrophied partner of a gastrocnemius pair. The computed average value for each muscle is encircled.

tracile power. The measurement of the birefringence and of the diameter of various fibers of an atrophied muscle revealed without exception that there exists no parallel between diminution of fiber diameter and loss in birefringence (fig. 2). In normal muscle, as is well known, the diameters of individual fibers differ distinctly, the thickest being often twice as thick as the thinnest. The variation in birefringence is much smaller. After atrophy, the fiber diameter often varies in the proportion 1:4, and the differences in birefringence increase appreciably. However, as seen from figure 2, there is no relation between loss in birefringence and diminished fiber diameter. A rather small fiber can still have a rather high bire-

fringence while a relatively thick fiber may have a much diminished birefringence.

Discussion. Hines and Knowlton (1937) have demonstrated that in atrophying muscles the relative amount of the contractile tissue, the "muscle cell phase," and of the non-contractile tissue, the "non-muscle phase," are altered. In a recent statistical study concerning the effect of atrophy upon the strength, these authors (Knowlton and Hines, 1940) expressed the tension values per unit "muscle cell phase." From a theoretical point of view, without doubt, this method is preferable to that used in this paper. However, the determination of the "muscle cell phase" depends on an exact measurement of the water and chloride content. Knowlton and Hines, in their statistical investigation for these determinations, used muscles from other animals but at the same stage of atrophy as those rats of which the muscle strength had been measured. Such a procedure could not be used in my experiments, where individual muscles were compared. In an attempt to take into account at least to a certain extent the shift in the two phases, the strength of the muscles was related to wet weight as well as to dry weight. One must keep in mind that a strict comparison of the water content of the partners of a muscle pair is impossible under the given conditions. The vascular system of denervated or trained muscles does not respond in the same manner to muscular activity as that of normal muscles. In general, the activity hyperemia in electrically trained muscles is larger, but subsides more quickly than in normal muscles (Vanotti and Magiday, 1934) while in the denervated muscles the circulatory responses are slow and irregular.

The difference in the methods of expressing the contractile power of the muscles is only partly the reason why in my experiments contractile power does not decrease before the weight loss of the denervated muscles amounts to about 30 per cent, while Knowlton and Hines (1940) report power losses when the weight loss reaches 20 per cent. This discrepancy is due probably mainly to the fact that these authors used for stimulation electrical shocks of uniform duration for all stages of atrophy, while in my experiments the length of the single shocks was increased with proceeding atrophy, so that optimal stimulation was obtained (Fischer, 1939).

The apparent tendency to an increase in contractile power during the first stage of atrophy (table 3) is probably due to a spreading effect and an incomplete fixation of the knee joints. A more rigid fixation than that used (i.e., clamps on the exposed femurs instead of clamps on the uninjured knee joints) would have interfered too much with the restoration of normal blood supply after cessation of stimulation. During the first days of atrophy, the difference between optimally shaped electrical shocks for atrophying muscles and for normal ones is relatively high concerning threshold but still small as to duration, so that in those days an optimal

stimulation of the atrophied gastrocnemius will have a marked spreading effect upon the non-denervated muscles of the thigh. With progressing atrophy, when the duration of the shocks must be rather long and their frequency low for optimal stimulation, the thigh muscles will be much less affected by the spreading effect.

The second series of experiments reported here confirms earlier authors (for literature see Steinhaus, 1933), who report that training with faradic currents produces a muscle hypertrophy due mainly to increased fiber diameter. Why in my experiments, despite the use of a training method which should be optimal from a theoretical viewpoint, some muscles did not distinctly gain weight, and why the maximal gain observed is far under the 40 per cent reported by others, is hard to explain. It might be due to the choice of the species and muscles employed. There are no indications that I overtrained the gastrocnemii by either too strong or too long a stimulation. That such an overtraining can occur for muscles stimulated electrically has been pointed out by Lehnartz (1936). However, all trained muscles of my rats, with or without appreciable weight gain, were distinctly less fatigable than their normal partners. Since in the trained muscles neither contractile power per weight unit nor birefringence increased, the various electrical training effects upon the chemical constituents of the muscle as reported at first by Embden and Habs (1927) and studied later mainly by Russian authors (Vanotti and Magiday, 1934; Rosengart, 1936, 1937; Palladin and Raschba, 1937; Klimenko, 1937; Werbolowitsch, 1937) are probably all connected with the decrease in fatigability.

The response of the normal muscle to faradic training with its increased weight but unchanged specific strength and birefringence resembles the response of atrophied muscles to electrical treatment. As shown in an earlier paper (Fischer, 1939) such a treatment retards distinctly the weight loss of the denervated muscles but is practically unable to delay the loss in contractile power or in birefringence. The comparison of the diminution of the diameter of individual atrophying fibers with their birefringence added further evidence in support of the suggestion advanced that weight loss and destruction of the submicroscopical pattern during atrophy are two distinct processes.

The observed statistical relation between birefringence of normal muscles and their contractile power as well as the parallel diminution of birefringence and contractile power of atrophying muscle is a further indication that the submicroscopical crystalline structure of the muscle is essentially involved in the contractile mechanism. Any attempt to explain the difference between the relation of birefringence to contractile power for normal muscle and for atrophying muscle can at the moment only be based on pure speculation with little relation to other experimental data, and would be outside the scope of this Journal.

SUMMARY

1. Electrical training of rat gastrocnemii increases their weight and their total isometric strength, but does not alter their contractile power per weight unit.

2. In denervated atrophying rat gastrocnemii, the loss in total isometric strength corresponds at first to the loss in weight, thus leaving the contractile power per weight unit unaltered. This early phase of atrophy, in which also no appreciable changes in birefringence are observed, lasts 9 to 11 days, and at its end the weight losses of the muscles have reached about 30 per cent. During the later phase of atrophy, the tension loss surpasses distinctly the weight loss. Birefringence starts to diminish too, and at about the same rate as the contractile power per weight unit. In consequence, the latter parallels the birefringence of the muscle throughout the whole course of atrophy. Even for the individual fibers, there exists no fixed relation between diminished fiber diameter and loss in birefringence.

3. For normal muscles, there exists a statistical relation between birefringence and contractile power per weight unit, which can be expressed by the equation: birefringence = k × log. contractile power.

4. The experimental data are discussed and the conclusion drawn that the submicroscopical crystalline structure of the muscle is essentially involved in the contractile mechanism.

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THE MECHANISM OF THE SECRETION OF ACID BY THE GASTRIC MUCOSA

HORACE W. DAVENPORT AND R. B. FISHER

From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York, and the Department of Biochemistry, The University of Oxford, Oxford, England

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Hollander (1934) and Gilman and Cowgill (1931) and others have shown that the acid secretion of the gastric mucosa is pure hydrochloric acid of concentration such that it is isotonic with the blood. In forming the acid secretion the cells of the gastric mucosa of dogs raise the hydrogen ion concentration from 5×10^{-8}, the hydrogen ion concentration of blood, to 0.17 M, the hydrogen ion concentration of the acid secretion. The chloride ion concentration is raised from 0.11 M in plasma to 0.17 M in the secretion. Osmotic work is done in raising the concentration of the two ions. The minimal amount of energy needed to do the work is equal to the free energy increase obtained in raising the concentration of the ions. The free energy change can be calculated by means of the equation used by Borsook and Winegarden (1931) in their calculation of the work of the kidney. It has been found that the gastric mucosa must expend a minimum of 772 small calories per liter of secretion in concentrating the hydrogen ions and a minimum of 48 small calories per liter in concentrating the chloride ions. Since the osmotic pressures of blood and gastric juice are the same the mol fraction of water in each is the same. Consequently no reversible work is done when water passes from the blood to the secretion, and no reversible energy is expended. A very small amount of irreversible work is done on the water in overcoming viscosity when water moves at a finite rate. The energy necessary to perform this work is doubtless derived from the difference in hydrostatic pressure between the blood and the gastric juice.

Davenport (1939, 1940a, 1940b) has suggested that the mechanism of the secretion of acid is that expressed in figure 1. Some mechanism in the parietal cells whose rate of action is directly proportional to the rate of formation of carbonic acid in the cells secretes hydrogen ions and uses energy. The principle of electrical neutrality of solutions requires that exactly the same number of anions be present in the secretion as there are hydrogen ions. In order to satisfy the principle, chloride ions pass

from the plasma through the cells and into the secretion, being dragged along by the positive charge on the hydrogen ions. The chloride ions removed from the plasma are replaced by bicarbonate ions formed in the cells at the same time the hydrogen ions are formed (Bulger, Allen and Harrison, 1928). Water moves through the cells and into the secretion without osmotic work being done on it.

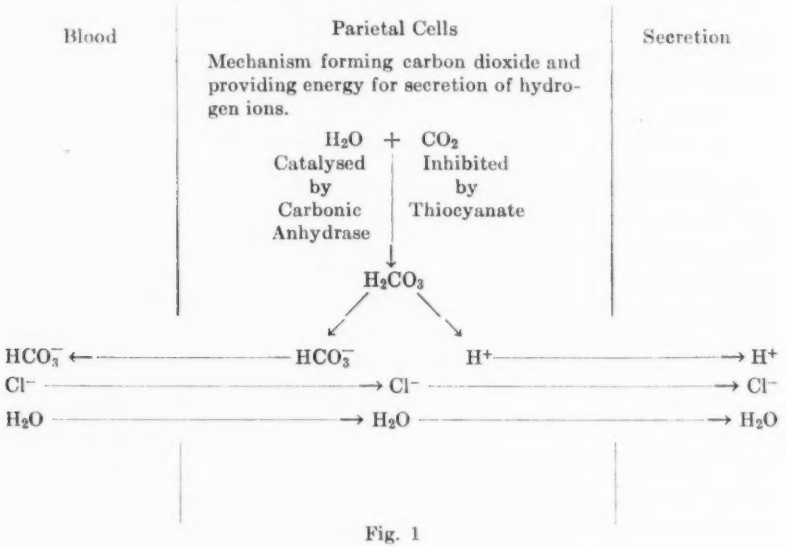

Fig. 1

The movement of chloride ions from the plasma to the secretion is in effect the transport of ions under the influence of an electrical potential difference. The velocity of the movement, u_{Cl}, is expressed by the equation

$$u_{Cl} = U'_{Cl} \frac{dE}{dl} \tag{1}$$

where dE/dl is the potential gradient and U'_{Cl} is the mobility of the ion in the cells. If it were possible to replace part of the chloride by another anion A^- the velocity of the anion, u_A, would be expressed by the equation

$$u_A = U'_A \frac{dE}{dl}. \tag{2}$$

Since the potential gradient is the same for each anion the relation between the concentrations of the anions in the plasma and gastric juice is expressed by the equation

$$\frac{[A^-]_{GJ}}{[Cl^-]_{GJ}} = \frac{[A^-]_P \; U'_A}{[Cl^-]_P \; U'_{Cl}} \tag{3}$$

where the left hand term is the ratio of the concentrations in the gastric juice and the right hand term is the ratio of the concentrations in the plasma multiplied by the ratio of the mobilities of the anions.

The mobilities of the anions in the cells and through the several membranes involved are unknown and cannot be measured. However it is reasonable to assume that they are directly proportional to the mobilities in water. This assumption is expressed by the equations $U'_A = k_A U_A$ and $U'_{Cl} = k_{Cl} U_{Cl}$ where U_A and U_{Cl} are the mobilities in water and k_A and k_{Cl} are constants. The constants express the retarding action of the cell contents and membranes upon the free movement of the anions. No doubt the magnitude of the constants depends upon the pore size of the membranes, the viscosity of the cells, the size of the anions and other factors. Since these factors can only be guessed at it is inadvisable to attempt any theoretical derivation of their magnitudes.

Substituting in equation (3) the equation

$$\frac{k_A}{k_{Cl}} = \frac{[A^-]_{GJ}\,[Cl^-]_P\,U_{Cl}}{[Cl^-]_{GJ}\,[A^-]_P\,U_A} \tag{4}$$

is obtained.

If the proposed mechanism of secretion be correct the observed ratio of k_A to k_{Cl} should be constant when the concentrations of A^- and chloride in the plasma are varied. If the anions A^- and Cl^- are retarded in passing through the cells to exactly the same extent the ratio should be equal to unity. If the anion A^- is retarded to a greater extent the ratio should be less than unity, and the degree of deviation from unity is a measure of the degree of retardation.

Equation (4) can be tested experimentally by the substitution of bromide for part of the chloride in blood. Quastel and Yates (1934) and Troenhart (1935) have shown that when bromide is present in the blood it is secreted into the gastric juice, but they made no attempt to test the mechanism of secretion in the way outlined here. Hoppe (1906) and Lipschitz (1929) found the ratio of bromide to chloride in the gastric juice to be approximately the same as that in plasma, but their data are insufficient for accurate analysis.

In the experiments reported in this paper bromide was administered to dogs. The concentrations of bromide and chloride in the gastric juice and plasma were determined. The values of the mobilities of bromide and chloride in water at various concentrations are given in Landolt-Bornstein (1923). They were plotted in the graph shown as figure 2, and the mobilities at the observed plasma concentrations were read off. The several observed quantities were substituted in equation (4), and the ratio of the constants was calculated.

It is well known that when bromide is administered it displaces chloride

from the plasma in such a fashion that the total concentration of bromide and chloride remains constant. In the animals used in these experiments $[Br^-]_P + [Cl^-]_P = 0.112$ M. Consequently for any given ratio $[Br^-]_P/[Cl^-]_P$ there are unique values of $[Br^-]_P$ and $[Cl^-]_P$, and those values can easily be calculated. By reading off the values of U_{Br} and U_{Cl} corresponding to the values of $[Br^-]_P$ and $[Cl^-]_P$ the ratio U_{Br}/U_{Cl} can be calculated for any plasma bromide to chloride ratio. By this means it was found that as the ratio $[Br^-]_P/[Cl^-]_P$ increases from 0.1 to 0.8 the ratio U_{Br}/U_{Cl} diminishes. Since it has been assumed that the actual mobilities in the secreting mechanism are directly proportional to the mobilities in water it should be found that as the ratio $[Br^-]_P/[Cl^-]_P$ increases the ratio U'_{Br}/U'_{Cl} diminishes. By substituting the observed plasma and gastric juice bromide and chloride concentrations in equation (3) the experimentally determined values of U'_{Br}/U'_{Cl} can be calculated, and the ratio should be found to diminish as the plasma bromide to chloride ratio increases. The observation of such a diminution is as important a confirmation of the theory as is the constancy of the ratio k_{Br}/k_{Cl}.

In the presentation and discussion of the results the ratio U'_{Br}/U'_{Cl} is denoted by the term *secretion ratio*, and the ratio k_{Br}/k_{Cl} is denoted by the term *secretion constant*.

METHODS. Bromide and chloride were determined by the potentiometric method of Hastings and van Dyke (1931). The method was carefully tested and found to be accurate to within 1 per cent. Thiocyanate was determined by the method of Lavietes, Bourdillon and Klinghoffer (1936). Since the solubility product of silver thiocyanate is almost identical with that of silver bromide thiocyanate is included in the titre of bromide in the potentiometric titration. When thiocyanate was present its independently determined concentration was subtracted from the apparent bromide concentration in order to obtain the true bromide concentration. The thiocyanate concentration was always small compared with that of bromide.

The gastric contents of dog 1 were sampled by means of a stomach tube. Dogs 2 and 3 were provided with Pavlov pouches. In the first group of experiments sodium bromide was added to the diets, and in the second group sodium thiocyanate was also added. At least 16 hours after feeding standard subcutaneous injections of histamine were given. During the second half-hour after injection samples of gastric juice were taken from dog 1, and samples were taken from dogs 2 and 3 as the juice was secreted. The rate of secretion by the pouch of dog 3 was measured and expressed as the number of milliliters of juice secreted during the second half-hour after injection. In that interval the rate of secretion was constant. Blood was obtained by venepuncture.

The secretion of bromide and chloride. The results obtained when bro-

mide alone was fed are presented in table 1 and in the first half of table 2 together with the calculated secretion ratios and constants. The relation of the secretion ratios and constants to the plasma bromide to chloride ratios are more clearly shown in figures 3a and 3b where they are plotted as filled circles.

The theoretical secretion ratio is equal to the ratio of the mobilities of bromide and chloride in water multiplied by the ratio k_{Br}/k_{Cl}, the secretion constant. The ratio of the mobilities in water for plasma bromide to

TABLE 1

DOG NUMBER	PLASMA			GASTRIC JUICE					SECRETION RATIO	SECRETION CONSTANT
	Cl^-	Br^-	$\frac{Br^-}{Cl^-}$	Free acid	Total acid	Cl^-	Br^-	$\frac{Br^-}{Cl^-}$	$\frac{U'_{Br}}{U'_{Cl}}$	$\frac{k_{Br}}{k_{Cl}}$
1	0.102	0.011	0.108	0.062	0.071	0.133	0.015	0.113	1.046	0.913
	0.089	0.026	0 292	0.067	0.074	0.120	0.036	0.300	1.027	0.929
	0.087	0.030	0.345	0.088	0.095	0.116	0.041	0.353	1.023	0.932
	0.082	0.029	0.354	0.083	0.092	0.118	0.043	0.364	1.028	0.936
	0.079	0.035	0.443	0.093	0.107	0.111	0.050	0.450	1.016	0.936
	0.072	0.043	0.598	0.064	0.109	0.108	0.064	0.593	0.992	0.924
	0.071	0.045	0.634	0.056	0.069	0.091	0.058	0.638	1.006	0.914
	0.068	0.046	0.677	0.053	0.064	0.090	0.060	0.667	0.985	0.925
2	0.093	0.016	0.172	0.134	0.138	0.143	0.026	0.183	1.064	0.947
	0.086	0.022	0.256	0.137	0.142	0.136	0.036	0.265	1.035	0.933
	0.091	0.026	0.286	0.113	0.128	0.131	0.038	0.290	1.014	0.916
	0.083	0.029	0.350	0.122	0.130	0.128	0.045	0.352	1.006	0.918
	0.080	0.033	0.413	0.129	0.132	0.122	0.051	0.418	1.012	0.930
	0.077	0.036	0.468	0.134	0.141	0.116	0.053	0.557	1.190	0.901
	0.074	0.040	0.540	0.122	0.132	0.110	0.060	0.545	1.009	0.936
	0.070	0.044	0.629	0.124	0.134	0.106	0.067	0.632	1.005	0.940
	0.064	0.046	0.719	0.126	0.134	0.098	0.070	0.714	0.993	0.936
	0.061	0.047	0.771	0.139	0.143	0.098	0.075	0.765	0.993	0.937
										0.928

All concentrations in mols per liter.

chloride ratios from 0.1 to 0.8 were calculated as explained above. These calculated ratios were then multiplied by the observed secretion ratio, 0.929. They were plotted as the curved line in figure 3a. The observed secretion ratios plotted as filled circles are obviously distributed at random about the theoretical line, and as the plasma bromide to chloride ratio increases the secretion ratio diminishes exactly as predicted by the theory. On the other hand the values of the secretion constant plotted as filled circles in figure 3b vary irregularly about their mean value, the deviations all lying within the narrow limits of 99.4 and 101 per cent of the mean

value of 0.929 ± 0.010. The data therefore establish without doubt that the secretion ratio diminishes as the plasma bromide to chloride ratio

TABLE 2

Uninhibited and inhibited secretion by dog 3

PLASMA				GASTRIC JUICE							SECRETION RATIO	SECRETION CONSTANT
Br^-	Cl^-	SCN^-	$\frac{Br^-}{Cl^-}$	Free acid	Total acid	Rate	Br^-	Cl^-	SCN^-	$\frac{Br^-}{Cl^-}$	$\frac{U'Br}{U'Cl}$	$\frac{k_{Br}}{k_{Cl}}$
0.011	0.100		0.110	0.133	0.147	14.9	0.018	0.156		0.115	1.045	0.920
0.012	0.100		0.120	0.132	0.145	15.1	0.020	0.156		0.128	1.067	0.941
0.018	0.093		0.194	0.131	0.139	15.0	0.030	0.146		0.205	1.057	0.945
0.019	0.094		0.202	0.119	0.135	14.2	0.034	0.144		0.208	1.030	0.921
0.019	0.093		0.204	0.132	0.144	15.2	0.031	0.145		0.214	1.049	0.940
0.019	0.092		0.207	0.133	0.141	14.5	0.030	0.141		0.213	1.029	0.921
0.021	0.090		0.233			14.2	0.034	0.142		0.240	1.030	0.927
0.028	0.082		0.341	0.133	0.143	15.8	0.045	0.129		0.349	1.023	0.934
0.029	0.081		0.358				0.045	0.124		0.363	1.014	0.925
0.031	0.082		0.378	0.135	0.144	14.9	0.049	0.126		0.389	1.029	0.942
0.036	0.077		0.468	0.139	0.148	16.8	0.057	0.122		0.467	0.998	0.921
0.038	0.073		0.521	0.132	0.145	16.0	0.060	0.116		0.517	0.992	0.919
0.038	0.071		0.535	0.132	0.140	15.0	0.061	0.115		0.530	0.991	0.920
0.039	0.071		0.549	0.121	0.131	15.8	0.062	0.113		0.549	1.000	0.930
0.043	0.068		0.633	0.135	0.143	15.2	0.068	0.109		0.624	0.986	0.923
0.046	0.065		0.708	0.128	0.140	16.1	0.073	0.103		0.709	1.001	0.941
0.048	0.063		0.762	0.133	0.143	15.2	0.076	0.101		0.752	0.987	0.931
				0.131	0.142	15.3 ±0.5						0.929
0.021	0.086	0.0033	0.244	0.111	0.123	12.5	0.034	0.131	0.0009	0.252	1.033	0.932
0.021	0.086	0.0044	0.244	0.103	0.121	7.8	0.033	0.129	0.0012	0.256	1.049	0.946
0.023	0.092	0.0018	0.250	0.117	0.127	12.4	0.034	0.135	0.0006	0.252	1.008	0.909
0.021	0.084	0.0042	0.250	0.109	0.119	13.1	0.034	0.131	0.0007	0.260	1.040	0.940
0.024	0.090	0.0037	0.267	0.095	0.106	9.2	0.035	0.127	0.0015	0.276	1.033	0.932
0.026	0.084	0.0042	0.310	0.093	0.113	8.2	0.038	0.121	0.0015	0.314	1.031	0.920
0.027	0.083	0.0042	0.325	0.060	0.075	5.4	0.038	0.114	0.0017	0.333	1.025	0.934
0.027	0.080	0.0036	0.338	0.117	0.127	10.6	0.040	0.118	0.0006	0.339	1.003	0.914
0.029	0.082	0.0025	0.354	0.125	0.135	14.3	0.044	0.122	0.0006	0.361	1.020	0.929
0.031	0.080	0.0011	0.388	0.125	0.133	16.4	0.047	0.119	0.0005	0.395	1.018	0.933
												0.929 ±0.011

All concentrations in mols per liter.

increases, and they demonstrate as well as can be expected that the secretion constant has a value constant within the limits of error and independent of the plasma bromide to chloride ratio. The figures further

illustrate the point that the data from all the dogs are entirely consistent, so that it may be concluded that the observed relation is a general one independent of individual variation or minor modifications in technique.

The observed constancy of the secretion constant over a wide range of concentrations clearly demonstrates that equation (4) is a correct statement of the mechanism of the secretion of bromide and chloride. Consequently it can be concluded that in all probability bromide and chloride are secreted by the same mechanism and that the mechanism acts on the ions through their negative charges in the manner postulated.

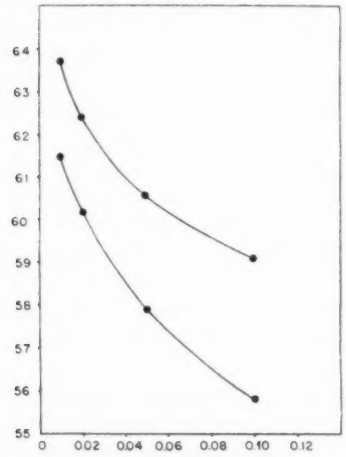

Fig. 2

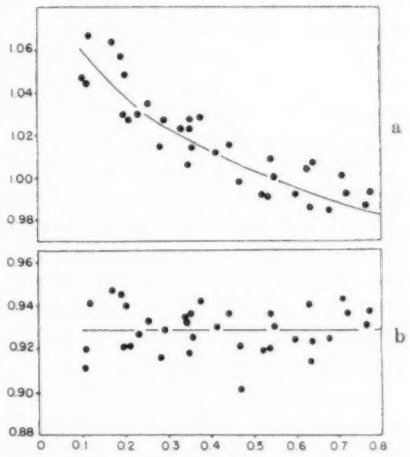

Fig. 3

Fig. 2. Ordinates: mobilities. Abscissae: molarity. Upper curve is the mobility of bromide in water; lower curve is the mobility of chloride in water.

Fig. 3a above. Ordinates: Secretion ratio. Abscissae: Ratio of bromide to chloride in the plasma.

Fig. 3b below. Ordinates: Secretion constant. Abscissae: Ratio of bromide to chloride in the plasma. Both figures are drawn to the same scale.

It may also be concluded that the assumption that the mobilities of the ions in the cells are directly proportional to their mobilities in water is correct. The secretion constant is a measure of the retardation of transport across the cells of the mucosa of bromide relative to chloride due to causes other than the differences in mobility of the ions in the cells. Since it is clear that such retardation might be expected to occur as a result of differences in the ease of passage across cell membranes it is reasonable to expect that it might bear a relation to the relative sizes of the ions. In this connection it is of interest to note that the ratio of the ionic radii of chloride and bromide derived from the data given by Pauling (1939) is

0.926 whilst the mean value of the secretion constant is 0.929. The correspondence between these values may be pure coincidence, and in any event there is no secure theoretical basis on which to found any expectation concerning the relation between ionic dimensions and transport across a cell membrane.

The data in table 1 and in the first half of table 2 also show that the acidity of the gastric secretion, the total halide concentration of the gastric secretion and the rate of secretion are entirely independent of the plasma bromide to chloride ratio. These facts strongly support the conclusion that the mechanism secreting acid is completely unaffected by the substitution of bromide for chloride. The acid secreting mechanism is indifferent to the chemical nature of the anion, and provided that an ion with a negative charge is present the mechanism is capable of secreting hydrogen ions at the same rate and at the same concentration as when chloride alone is present.

Inhibition by thiocyanate. Davenport (1940b) has shown that the carbonic anhydrase in the parietal cells and the secretion of acid are inhibited by thiocyanate ions. He postulated that the parietal cell carbonic anhydrase catalyses the hydration of carbon dioxide to carbonic acid and that the ionization of the carbonic acid provides the hydrogen ions for the acid secretion. When the rate of hydration of carbon dioxide is reduced by inhibition of the enzyme the rate of secretion of hydrogen ions is proportionately reduced.

The secretion of chloride ions is also reduced. According to the theory outlined in this paper the reason for the reduction of chloride secretion is that the diminution of the production of hydrogen ions reduces the number of positive charges in the secretion. The fewer hydrogen ions secreted the fewer anions are needed to maintain electrical neutrality of the secretion. Since the inhibition has no direct effect upon the secretion of the anions there should be no difference between the relation of bromide to chloride during normal and during inhibited secretion. Consequently it would be predicted that during inhibition the secretion ratio would bear the same relation to the plasma bromide to chloride ratio and that the secretion constant would be identical with that found during uninhibited secretion. However the total halide concentration of the secretion should be reduced. If on the other hand this theory be wrong in that the secretion of chloride is effected by some active mechanism acting directly on the chloride it would be expected that the inhibition of secretion would greatly upset the relation of bromide to chloride. Therefore the secretion ratio and secretion constant should differ from those found during uninhibited secretion.

These predictions were tested by the administration of bromide and chloride together with thiocyanate to dog 3. Bromide and thiocyanate together are more toxic than either alone, and only the lower bromide to

chloride ratios could be attained. The results are presented in table 2, and they can be compared with control observations on the same dog.

The results clearly show that when thiocyanate is present the acidity and the total halide concentration of the secreted juice and the rate of secretion are reduced. These results agree with the theory developed by Davenport (1940b) upon the postulate that as a first approximation the rate of secretion of acid is directly proportional to the rate of formation of carbonic acid in the parietal cells. However the secretion ratio varies with the plasma bromide to chloride ratio in exactly the same manner as when the secretion is not inhibited. The mean value of the secretion constant, 0.929 ± 0.011, is identical with the value 0.929 ± 0.010 obtained in the control experiments.

These results prove that the relation of bromide to chloride in the gastric juice is a function only of the relation of bromide to chloride in the plasma and of the physico-chemical properties of the anions. That relationship is not disturbed by very considerable diminution of the rate of secretion of hydrogen ions. Thiocyanate does not interfere directly with the secretion of the anions, and the total halide concentration of the gastric juice is reduced only because the secretion of hydrogen ions is reduced. These facts very strongly support the theory of acid secretion outlined in this paper, and it can be concluded that the passive character of the secretion of the anions is fully established. Further work on the mechanism of acid secretion must endeavor to explain fully the means by which the hydrogen ions are concentrated and secreted.

Conclusion. The results show that the concentration of bromide in the gastric secretion is always greater than the concentration in the plasma. In the observations on dogs 1 and 2 and in the uninhibited observations on dog 3 the ratios of bromide in the gastric juice to bromide in the plasma are on the average 1.38, 1.55 and 1.60 respectively. This means that in secreting a mol of bromide the gastric mucosa of dog 1 expended a minimum of 199 small calories, dog 2 expended a minimum of 270 small calories, and dog 3 expended a minimum of 289 small calories. Only two explanations of this fact are possible. Either the gastric mucosa contains a mechanism specifically fitted for doing work in the secretion of bromide which is called upon only when concentrations of bromide enormously in excess of the normal are experimentally produced, or the mechanism secreting chloride is capable of switching over to the secretion of bromide at any concentration with only a small loss in efficiency. The second possibility is more probably the true one. Since the chemical properties of bromide and chloride, aside from the similarity of their negative charges, are so different it is again more likely that the mechanism acting on chloride acts on the ion through the negative charge in the manner postulated and is hence also capable of acting on bromide through its negative charge.

The energy used in concentrating bromide and chloride can easily be

supplied by the mechanism secreting hydrogen ions. A minimum of 772 calories is required to secrete the hydrogen ions in a liter of acid secretion. If the hydrogen ions must pull an equal number of anions along with them the energy used in attaining the high hydrogen ion concentration is in part also used to raise the concentration of the anions. Consequently the expenditure of 820 calories on the hydrogen ions in forming one liter of secretion would result in raising the potential energy of the hydrogen ions by 772 calories and of the anions by 48 calories.

In conclusion we particularly wish to emphasize that we demonstrate in this paper the passive character of the transport of bromide and chloride from plasma to gastric juice, and that we demonstrate this passivity by showing that the two anions behave differently, the differences in behavior being explicable in terms of those physico-chemical properties of the ions which would be expected to be involved in their passive translocation. These experiments can be taken to support the view that the active process in the secretion of hydrochloric acid by the gastric mucosa is the secretion of hydrogen ions.

We are indebted to Drs. E. S. Nasset, W. B. Hawkins and S. C. Madden for preparing the Pavlov pouches.

SUMMARY

Bromide is secreted by the gastric mucosa. Over a wide range of concentrations of bromide in the plasma the rate of secretion of bromide is of the same order as the rate of secretion of chloride.

The concentration of bromide and chloride in the gastric juice is always greater than that in the plasma. Osmotic work is done in concentrating chloride, and it is concluded that the work is performed on the two anions by the same mechanism.

The rate of secretion of the gastric juice and the acidity and total halide concentration of the secretion are completely independent of the plasma bromide to chloride ratio.

The apparent mobilities of bromide and chloride in the cells of the gastric mucosa are directly proportional to their mobilities in water.

When the rate of secretion of the gastric juice and the acidity and total halide concentration are reduced by inhibition by thiocyanate the relation of bromide to chloride in the gastric juice remains exactly the same function of the plasma bromide to chloride ratio as it is during uninhibited secretion.

It is concluded that the mechanism concentrating and secreting bromide and chloride acts only through the negative charges on the ions.

An incomplete theory of the mechanism of the secretion of hydrochloric acid by the gastric mucosa is proposed.

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RESPIRATION AND GLYCOLYSIS OF RABBIT BONE MARROW IN SERUM IN RELATION TO CELLULAR COMPONENTS[1]

CHARLES O. WARREN

From the Department of Physiology, Cornell University Medical College, New York City

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A recent publication (1) has described the considerably higher and more constant rates of respiration and glycolysis of slices of rabbit bone marrow obtained when serum rather than Ringer solution is used as the suspension medium. In an earlier study (2) dealing with the oxygen consumption of rabbit bone marrow in relation to its morphology, the measurements were made in Ringer solution, and glycolysis was not studied. The present paper reports further work on the metabolism of rabbit bone marrow in relation to its cellular composition, using the improved serum technique and extending the investigation to include aerobic and anaerobic glycolysis.

The rate of respiration has been measured by the "neutralized" serum technique (1) and, where necessary, these figures have been corrected for the oxygen consumption of the serum itself, which is occasionally large enough to introduce serious errors (cf. 3 and 4). Anaerobic glycolysis in serum has been measured manometrically, allowance being made in the usual manner for the retention of CO_2 in the serum. Aerobic glycolysis has been measured in Ringer-bicarbonate-glucose solution, since the methods for doing this manometrically in serum are complicated at best (cf. 1). Even with this simplified method, one assumes that the R.Q. is unity, and as this is probably not the case, this measurement will be referred to as the "apparent aerobic glycolysis" and considered as a rough index of the aerobic glycolytic activity of the tissue without implying that it is an accurate measurement of the true aerobic glycolysis. Since the R.Q. is usually less than unity, the "apparent aerobic glycolysis" will be smaller than the true value, but useful in following relative changes.

The Q values are expressed in terms of fat-free dry weights, calculated from nitrogen determinations as previously described (1). All experiments are conducted at a temperature of 37.5°C.

Cell counts are made on smears of each marrow, prepared as described previously, except that instead of grinding the marrow slices lightly in a

[1] Supported by a grant from the Committee on Scientific Research of the American Medical Association.

mortar before smearing, the slices are now suspended in a small quantity of serum and cut finely with scissors, and Wright-Giemsa stain is now used instead of Jenner-Giemsa. At least 1500 cells are counted in each case and divided into two classes—red, or erythroid, and white, or myeloid, and the percentage of immature cells in each series is also determined. This is done by selecting a stage of development in each series, and counting cells older than this as mature and younger ones as immature. In the case of the myeloid cells the dividing line is the Class I myelocyte of Sabin and Doan (6), which is itself counted as immature, and in the red cell line, any cell which contains hemoglobin is counted as mature. These criteria of maturity have been found to be more convenient than those used in the earlier paper, but in all other respects the remarks made at that time with respect to accuracy of counts, etc., apply to the present work.

In the earlier publication, the proportion of the various cell types in the marrow was altered by selecting animals of different age, but the changes in the cell population were not as large as desirable and in the present study more drastic means have been employed to accomplish this end. These procedures will be described below, but it is to be noted that the age of the animal has been held relatively constant, in that all the rabbits used were mature—they weighed between 3 and 4 pounds and occasionally more. The rabbits were all New Zealand White males of a uniform strain; they received the usual laboratory diet of pellets supplemented occasionally with greens and were not fasted before being sacrificed for the experiments.

Results. I. *The time element in the experiments.* Before proceeding with the main problems under investigation, it was considered desirable at the outset to inquire whether or not the time elapsing between the death of the animal by venesection and the beginning of the metabolic measurements was a factor influencing the results. Seven experiments were performed on different occasions in which the marrows were very rapidly sliced and placed in vessels which had already been equilibrated in the water bath. By this means it was possible to reduce the time necessary for further equilibration and the readings were started after an average interval of 48 minutes following the death of the animal. Second and third samples of the same marrows were allowed to stand in Ringer solution at room temperature, either sliced or unsliced, for further one and two-hour periods respectively before being placed in the bath, and a fourth group was allowed to stand at room temperature for an average interval of four hours after killing the animal. If the rate of respiration of the first group is considered as 100 per cent, that of the second group averaged 102 per cent, of the third group 98 per cent and of the fourth group 92 per cent. Consequently, such decrease in rate of respiration as may take place after killing the animal either occurs during the first 48 minutes (and hence is not detected by these methods) or not until between $2\frac{3}{4}$ and 4 hours at room

temperature. In all the following experiments the readings were always begun within 2½ hours after killing the animal, so that the influence of the time element, if any such exists, has been eliminated to as great an extent as possible. Similar experiments demonstrate that the same statement also applies to apparent aerobic and to anaerobic glycolysis.

Also, the morphological changes in the marrow occurring *in vivo* after death, as described by Rohr and Hafter (7), do not occur when the marrow is removed from the animal immediately after death and placed in Ringer solution. In fact, following respiration experiments in neutralized serum

TABLE 1

Respiration, glycolysis and cell counts of normal marrows

EXP. NO.	(1) Q_O	(2) $Q_G^{O_2}$	(3) $Q_G^{N_2-CO}$	(4) (3)/(1)	(5) PER CENT MYELOID CELLS	(6) PER CENT MYELOID CELLS IMMATURE	(7) PER CENT ERYTHROID CELLS IMMATURE
85	−6.3	0.6	11.5	1.82	50	10	9
86	−5.9	2.2	13.0	2.20	47	12	17
88	−6.0	2.1	13.7	2.28	53	9	10
89	−6.7	2.7	15.3	2.28	50	11	15
90	−7.6	3.1	14.7	1.94	43	10	10
91	−6.0	4.0	12.2	2.04	47	17	16
92	−5.9	2.6	11.3	1.92	40	10	10
93	−5.4	2.6	12.4	2.28	48	16	11
94	−7.2	4.3	14.3	2.00	51	13	29
95	−7.8	3.5	15.9	2.00	44	14	14
96	−6.7	4.1	16.0	2.40	66	9	10
97	−6.4	1.7	11.4	1.78	32	10	18
N-5	−5.8	2.6	13.7	2.38	56	5	10
N-6	−6.2	1.6	12.7	2.06	43	12	15
N-7	−6.7	1.9	15.5	2.31	48	8	14
N-8	−8.0	3.3	16.2	2.02	57	9	17
N-9	−6.3	2.7	15.9	2.52	56	6	14
N-10	−7.0	1.4	13.2	1.90	41	12	18
Av.....	−6.6	2.7	13.8	2.09	48.5	10.7	14.3

in which the marrow has been shaken in the vessels for more than 4 hours, the cells are morphologically indistinguishable from those stained immediately after killing the animal. In the meantime, the rate of respiration has been practically constant with respect to time, as previously described (1), but in the case of glycolysis, the rate may begin to decrease after the first hour, so that only the first hour readings have been used in calculating the results given in Table 1.

II. *Normal marrows.* Table 1 shows the results obtained with 18 normal marrows. The first 3 columns indicate the rates of respiration,

apparent aerobic and anaerobic glycolysis respectively, each expressed in the conventional terms, based, however, on the fat-free dry weights calculated from nitrogen determinations. The experiments are shown individually in order that the variations between different marrows may be noted. The variation is over a fairly wide range, as is not unusual in this type of study, but it is noted that in general, in those instances in which the rate of respiration is unusually high, the rate of anaerobic glycolysis is also high, so that the ratio between these two entities (column 4) varies within a smaller range than either entity alone. Its average value is about 2.1 for normal marrows. The apparent aerobic glycolysis (column 2) shows particularly wide variations; the figures are given only to indicate that normal rabbit bone marrow does exhibit an appreciable aerobic glycolysis.

Histologically, these marrows are characterized by the myeloid and erythroid cells appearing in about equal numbers (column 5). This is a higher proportion of erythroid cells than is usually found in normal rabbits (2) (6) and is apparently a characteristic of this particular strain. The erythroid cells, however, are smaller than the myeloid cells (2), so that in these normal marrows the mass of myeloid cells is greater. There are relatively few immature cells in either series; the fact that there appear to be more immature erythroid than myeloid cells is without significance since it is due merely to the different criteria of "immaturity" chosen for the two series.

III. *Erythroid marrows.* Two groups of rabbits were treated in such a way that erythrogenesis was simulated and erythroid cells considerably outnumbered the myeloid cells in the marrow. In the first group, the animals were bled by heart puncture several times a week for various periods extending in some cases over a month. Successively large amounts of blood were withdrawn, beginning with about 20 cc. and ending with about 40 cc.; the plasma was discarded, the red cells hemolyzed with distilled water and the hemolysate reinjected into the animals intraperitoneally. These procedures induce a severe anemia and at the same time supply the materials whereby the marrow can replace the blood loss. As has been shown by Miller and Rhoads (8), the marrows come to exhibit a marked erythroid hyperplasia and the cell counts in the hemorrhagic group in table 2 indicate a corresponding decrease in the proportion of myeloid cells when compared with the normal marrows. The accompanying metabolic changes (first 4 columns) are seen to be a decrease in the rate of glycolysis, both aerobic and anaerobic, and a slight increase in the rate of respiration. The ratio between glycolysis and respiration (column 4) is therefore reduced from the normal value of 2.1 to about 1.6.

In a second group of animals a hemolytic anemia was induced by intraperitoneal injections of phenylhydrazine, usually 5 cc. of a 1 per cent solu-

tion for two doses with an intervening day, followed by an interval of several days before sacrificing the animals. Dickens has shown (9) that in addition to causing a hemolytic anemia, phenylhydrazine has a direct action on tissue metabolism characterized by an increase in aerobic glycolysis. Accordingly, the low aerobic glycolysis in the present experiments is evidence that the results are not due to a direct action of the drug on the marrow. It is also to be noted that the marrows were bright red and not brown as would have been the case if the phenylhydrazine had acted directly. The marrows showed the same morphologic and metabolic changes as in the first group but to an even greater extent, except that the rate of respiration, though averaging a little higher than normal, was not

TABLE 2

Respiration, glycolysis and cell counts of erythroid marrows

	EXP. NO.	(1) Q_{O_2}	(2) $Q_G^{O_2}$	(3) $Q_G^{N_2-CO}$	(4) (3)/(1)	(5) PER CENT MYELOID CELLS	(6) PER CENT MYELOID CELLS IMMATURE	(7) PER CENT ERYTHROID CELLS IMMATURE
Hemorrhagic group	E-6	−8.2	1.2	12.6	1.53	27	8	22
	E-7	−7.6	2.1	11.1	1.46	32	4	14
	E-8	−9.1	2.1	13.7	1.50	21	11	14
	E-9	−5.8	1.9	10.7	1.84	40	4	24
	E-10	−8.8	2.7	14.3	1.62	37	7	40
	Av.	−7.9	2.0	12.5	1.58	31	7	23
Phenyl hydrazine group	PH-2	−6.8	0.9	9.6	1.41	30	7	29
	PH-3	−6.2	1.1	7.1	1.14	18	9	43
	PH-4	−8.5	1.4	11.2	1.32	17	5	24
	PH-5	−7.2	0.7	9.0	1.25	9	9	20
	Av.	−7.2	1.0	9.2	1.28	19	8	29

quite as high as in the first group. This was more than counter-balanced, however, by the greater decrease in anaerobic glycolysis, so the ratio of the two fell to 1.3. In both groups of erythroid marrows glycolysis is evidently playing a relatively less active rôle than respiration in comparison with the state of affairs existing in normal marrows.

IV. *Myeloid marrows.* In these experiments, a wider variety of procedures was used to induce a myeloid hyperplasia in the marrows, and since in several instances more than one method was used with the same animal, there is considerable overlapping between the groups, as shown in table 3. The first group received successive large injections of saline intraperitoneally, inducing an exudation of white blood cells. Usually these were

left to degenerate or to be reabsorbed as the fluid is excreted, but in some cases the exudates were withdrawn. In any case, as is well known (10, 11), the marrow is stimulated to produce more myeloid cells. Three of these animals also received subcutaneous injections of sodium nucleinate, 25 mgm. a day, and two others received the sodium nucleinate alone, for about 20 doses. The studies of Doan and his collaborators (12, 13, 14) would lead one to suspect that this substance might have a direct effect on the marrow metabolism, but when added in high concentration directly to the marrow slices in the vessels, it did not alter their metabolism. The

TABLE 3

Respiration, glycolysis and cell counts of myeloid marrows

	EXP. NO.	(1) Q_{O_2}	(2) $Q_G^{O_2}$	(3) $Q_G^{N_2-CO}$	(4) (3)/(1)	(5) PER CENT MYELOID CELLS	(6) PER CENT MYELOID CELLS IMMATURE	(7) PER CENT ERYTHROID CELLS IMMATURE
Exudate group	M-1	−8.6	4.1	22.4	2.60	62	17	10
	M-3	−6.9	3.1	14.6	2.12	58	10	16
	M-8	−7.1	6.6	19.6	2.78	75	8	12*
	M-9	−6.7	5.0	15.0	2.24	62	11	10*
Sodium nucleinate group	M-6	−6.0	7.5	19.1	3.20	80	6	13
	M-10	−4.8	4.7	16.9	3.55	88	6	15
	M-12	−5.8	4.2	16.3	2.80	66	10	17
	M-11	−5.2	2.1	16.4	3.14	70	7	20
	M-13	−5.3	3.7	15.2	2.84	60	6	10
Infected group	M-5	−5.9	2.7	13.4	2.26	53	6	22
	M-7	−6.7	4.9	16.2	2.40	60	8	14
	A-2	−5.4	5.8	15.6	2.90	71	9	9
	A-3	−5.9	4.3	14.0	2.40	57	10	13
Average		−6.2	4.5	16.5	2.66	66	9	14

* Also had abdominal wall abscesses.

other animals were given chronic infections—2 an experimentally-induced arthritis, 2 had staphylococcus abdominal-wall abscesses, and 2 had scabies. The number of animals in each group is too small to permit conclusions as to the relative efficacy of these various procedures in inducing myeloid hyperplasia, but the best results were obtained by a combination of two methods, as indicated.

The cell counts in table 3 show that in practically all instances the myeloid hyperplasia consisted of increases in the relatively mature myeloid cells, so that the proportion of immature forms was not greater than nor-

mal. As was to be expected, the percentage of immature erythroid cells was not significantly changed. The accompanying metabolic changes are in the reverse direction from those found in the erythroid marrows—respiration tends to be slightly lower than normal and both apparent aerobic and anaerobic glycolysis are markedly increased. The average ratio between anaerobic glycolysis and respiration is raised to 2.7, indicating in these myeloid marrows a relative increase in the glycolytic activity with respect to respiration.

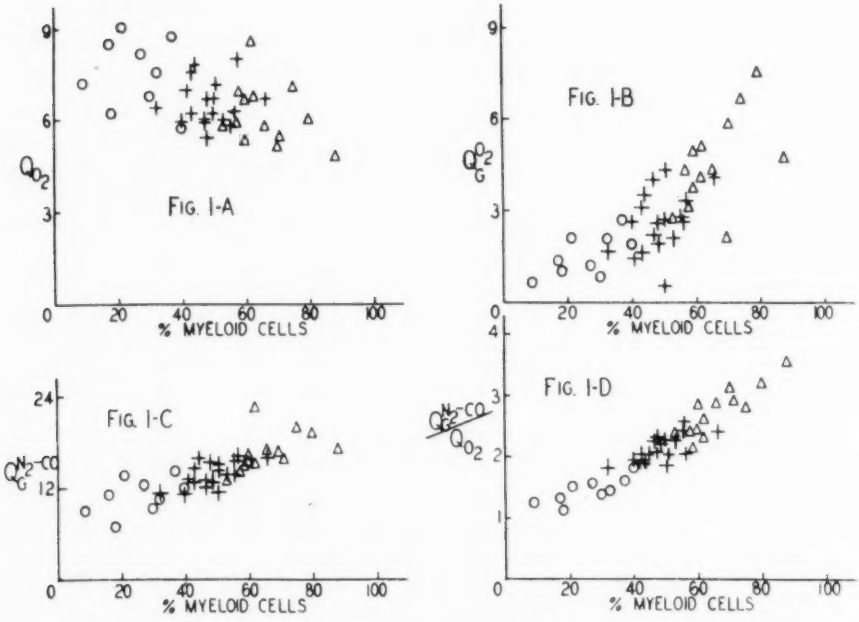

Fig. 1. Relation between respiration, glycolysis and cell counts. + Normal marrows. ○ Erythroid groups. △ Myeloid groups.

DISCUSSION. In figure 1 the metabolic data presented in the three preceding tables are reassembled and plotted with respect to the per cent of myeloid cells. Respiration, apparent aerobic and anaerobic glycolysis are represented in figures 1–A, B and C respectively, and in figure 1–D the ratio between anaerobic glycolysis and respiration, plotted in each instance against the percentage of myeloid cells. It is clear that in the first three instances the spread of the data is very wide, so that one can speak only of trends which are followed rather than of precise relationships. Thus, in the case of respiration, there is a trend towards slightly lower values as

the percentage of myeloid cells increases, whereas in the case of aerobic and anaerobic glycolysis the trends are clearly upwards. The ratio between anaerobic glycolysis and respiration, however, shows much less variation with respect to a given percentage of myeloid cells than any one of the other individual metabolic characteristics. Accordingly, this ratio is the most useful index by which the metabolism of the marrow may be characterized—it is in the neighborhood of 2.0 for normal marrows, between 1.0 and 1.5 for markedly erythroid marrows, and in the neighborhood of 3.0 for markedly myeloid marrows. Burk (15, p. 445) has emphasized the usefulness of this particular ratio in classifying tissues generally, and tumors in particular. The relation between tumor metabolism and bone marrow metabolism will be discussed a little further on.

One may inquire whether it is possible, from these studies, to estimate the results to be expected if suspensions could be obtained consisting entirely of erythroid or myeloid marrow cells. To do this, one would have to plot lines through the points in the figures and extrapolate them to the ordinate values for 100 per cent erythroid cells and 100 per cent myeloid cells respectively. Because of the spread of the data, one obviously cannot do this with precision, but since the trends are clear, it is perhaps justifiable to make rough approximations of the general levels of the metabolism in each case. These estimates are presented in table 4. It must be pointed out that the "typical" erythroid and myeloid cells referred to in the above table would be cells of intermediate stages of maturity, not megaloblasts or myeloblasts. In fact, the marrows studied in these experiments did not contain high enough proportions of relatively immature cells of either series to enable one to establish a relation between the metabolism of the cells and their maturity.

TABLE 4

Estimates of respiration and glycolysis of erythroid and myeloid cells

	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2-CO}$
Typical erythroid cell	High moderate (approx. −9)	Very low (nearly 0)	Low (approx. 7)
Average normal marrow	Moderate (approx. −7)	Moderate (approx. 3)	Moderate (approx. 14)
Typical myeloid cell	Low moderate (approx. −6)	High (approx. 9)	High (approx. 22)

In this connection, however, it should be noted that Kempner (16) has found that the myeloblasts of human myeloblastic leukemia exhibit no

aerobic glycolysis, whereas in the present study, the normal rabbit myelocytes have a relatively high rate of aerobic glycolysis, as do leukemic myelocytes (16, 17). Whatever may be the significance of these differences, it seems desirable to emphasize that the aerobic glycolysis of rabbit bone marrow in the present experiments is not to be interpreted as due to cellular damage as considered by Fleischmann (18) and Kempner (16). It persists in serum (1), is independent of the time element in the experiments, and is merely an index, although a rough one, of the proportion of myeloid cells in the marrow. *All* the aerobic glycolysis of the marrow is probably not due to the myeloid cells, since mature erythrocytes are well known to form small amounts of lactic acid aerobically (19, 20), and some of these cells are always present in the marrow, but certainly most of the aerobic glycolysis must be due to the normal myelocytes.

It is of interest that the figures given in table 4 for the metabolism of myeloid cells closely resemble those of the same sort for tumor cells, and that indeed the ratio of anaerobic glycolysis/respiration of nearly 4.0 is also characteristic of tumor metabolism (cf. Burk (15) p. 439, column VII divided by 3). The R. Q. of these myeloid cells has not been determined, and will be important to know, but the fact that these normal myeloid cells exhibit metabolic characteristics so closely resembling those of tumor cells is merely further evidence of the impossibility of making rigid distinctions between normal and cancer cells based on such metabolic evidence alone.

In general, the moderately high rate of respiration of the erythroid cells is in line with the findings in the earlier publication (2) as well as with the studies of Orr and Stickland (5), Michelazzi (21), v. Breza (22), Schretzenmayer and Brocheler (23) and Kempner (24), but the absolute level of the Q_{O_2} in some of these studies is quite different, probably due to differences in method, species differences, and differences in the relative maturity of the particular cells being studied. In the first paper of this series (2), it was found that the rate of respiration of the marrow increased as the percentage of myeloid cells increased. However, when these rates are recalculated on the basis of marrow fat-free dry weights, this relationship is found not to apply, and it seems clear (table 4) that the myeloid cells have, if anything, slightly lower rates of respiration than the erythroid cells.

The relatively high rates of aerobic and anaerobic glycolysis of the myeloid cells is in agreement with the findings of Orr and Stickland (5) who also worked with rabbit bone marrow, and with many studies (cf. 16) on blood leucocytes. But here again, quantitative comparison of leucocyte and marrow studies is not possible at present because of the number of variables involved.

Finally, mention must be made of the significance to be attached to the

finding that the myeloid cells possess relatively active glycolytic mechanisms whereas in the erythroid cells oxidative processes predominate. The predominance of oxidative processes in the erythroid cells raises two interesting questions: 1, is the synthesis of hemoglobin necessarily an aerobic process, and 2, by what mechanism does low oxygen tension stimulate the production of cells which are relatively dependent upon oxygen for their metabolic needs?

CONCLUSIONS

1. When slices of rabbit bone marrow are suspended in serum, the ratio between the rates of anaerobic glycolysis ($Q_G^{N_2-CO}$) and respiration (Q_{O_2}) is more constant for marrows of given cellular composition than either component of the ratio alone. The relationship between this ratio and the cellular composition of the marrow has been established; the ratio is relatively high for myeloid marrows and low for erythroid marrows. Myeloid cells are therefore characterized by possessing relatively active glycolytic mechanisms, and erythroid cells by the relative predominance of oxidative over glycolytic processes.

2. Estimates have been made of the results to be expected if suspensions could be obtained consisting entirely of myeloid or erythroid marrow cells. These are, in approximate figures:

	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2-CO}$
For erythroid cells	−9	0	7
For myeloid cells	−6	9	22

3. The significance of these results is discussed, and it is pointed out that the aerobic glycolysis of bone marrow is not to be considered as evidence of damage to cells, or of a tumor type of metabolism, but merely as an expression of its content of myeloid cells.

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AMMONIA FORMATION IN THE AMPHIBIAN KIDNEY[1]

ARTHUR M. WALKER

From The Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

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The formation of ammonia by the mammalian kidney is an established function (1) and an important one in regulating the neutrality of the blood. The experiments here to be described, first determining that the amphibian kidney shared in this ability to form ammonia, were designed to determine the site of this formation. Fluid was collected from various levels of individual nephrons and analyzed. The concentrations of ammonia in glomerular fluid and fluid from all levels of the proximal tubule were too low to be measured by the analytical method employed. Only after passage through a portion of the distal tubule did significant amounts of ammonia appear in the tubule fluid, and the latter portions of this segment are therefore the site of ammonia formation. Indications were obtained that the ammonia is formed from some precursor, stored within the tubule cells.

METHODS. a. *Collection of fluid.* Frogs (*R. pipiens* and *R. catesbiana*) were found to excrete ammonia in adequate concentration under the necessary experimental conditions and were used in the majority of experiments. Necturi (*N. maculosus*) excrete ammonia in low concentrations which, during tubule fluid collection, frequently diminish to a level that made analysis impossible or uncertain. Measures calculated to produce an acidosis did not help this situation.

Fluid from the proximal segment of the frog's tubule was collected by the technique described by Richards and Walker (2). Any suitably distended segment of tubule visible on the ventral surface of the kidney was punctured and fluid collected. The relation of site of puncture to the entire tubule was subsequently determined by injecting 0.1 per cent methylene blue through the same puncture hole and observing the course which it followed along the lumen. The identification is not exact but allows decision as to the quarter of the segment punctured.

Collections from the distal tubule of the frog were more difficult; partly because of the smaller amounts of fluid within this segment, chiefly because it rarely reaches the ventral surface to become available for puncture.

[1] The expenses of this work were defrayed in large part by a grant from The Commonwealth Fund.

The occasional segments reaching the surface were visualized by two methods: 1. Methylene blue was injected into a proximal tubule and its route observed as it flowed through the distal tubule until some portion of this tubule could be seen on the kidney surface. 2. The retrograde injection of small amounts of air into the tubules from a ureteral cannula allowed visualization of any distal segments which were on the kidney surface; the position of these tubules was noted and one was punctured after the air had flowed back again into the ureter. Collections from the extreme end of the distal tubule proved impossible because its junction with the collecting duct occurred well below the kidney surface.

In the course of these experiments it became necessary to collect fluid from the collecting ducts and from the upper reaches of the ureter. To accomplish this, the dorsal surface of the kidney was exposed and brought into the microscopic field. The ureter was obstructed by a clamp placed midway between the upper and lower halves of the kidney, a pipette was inserted into it above the obstruction, and sufficient air was injected to fill the ureter and move backward into the collecting ducts. The ducts were then seen as single or branching tubes entering the ureter at irregular intervals that averaged about 1 mm. They are considerably larger than tubules and the flow of fluid down them is relatively rapid. They rarely occupied a position on the kidney surface but when such an one was observed its position was marked by neighboring chromatophores, the ureteral clamp was removed, the air allowed to reënter the ureter and the puncture performed. Collections of fluid from the ureter presented no difficulties.

Contamination of the collected specimen by fluid sucked backwards from segments of tubule distal to the site of collection was avoided by the injection of a short column of mineral oil saturated with Sharlach-R to increase its visibility. Rate of collection, determined by the rate of flow within the tubule and pressure within the collecting tip, was adjusted to keep the oil column stationary at a point just distal to the tip of the pipette. In collecting glomerular fluid we have found the use of an oil column preferable to an obstructing rod (3) but both oil and rod are necessary in experiments upon the collecting ducts because of the rapid rate of flow within these structures.

b. *Analyses of fluid.* An ultramicro modification[2] of the direct Nesslerization method has been employed which, utilizing the technique of capillary tube colorimetry described by Richards, Bordley and Walker (4), permits quantitative analysis upon 0.1 c.mm. of fluid containing as little as one-millionth of a milligram of ammonia nitrogen. The procedure involves the introduction into a glass capillary tube with inner diameter

[2] Preliminary work in the development of this method was done by Dr. Charles L. Hudson in this laboratory in 1932.

0.35 mm. of 3 separate columns of fluid of measured length separated by columns of air; a 5 mm. column of the solution containing ammonia, a 2.5 mm. column of 2 per cent gum ghatti and, last, a 2.5 mm. column of Nessler's solution without added alkali. The columns are mixed by centrifugation and the color which develops is examined by placing the capillary upon the unglazed surface of a milk glass plate in bright skylight. In the event that sufficient unknown solution is not available to provide a column of 5 mm. in length, the amounts of the reagents employed are proportionately reduced. In determining the value of an unknown solution, capillary tubes are similarly charged with ammonium chloride solutions in concentrations of 0.5, 1.0, 2.0, etc., up to 10 mgm. per cent, mixture in the unknown and standard tubes is made simultaneously by centrifugation, and the color comparison made immediately thereafter while

TABLE 1

Determinations of ammonia nitrogen in the bladder urine of frogs

CONCENTRATION OF SOLUTION		DIFFERENCE
Found	Known	
mgm. per cent NH_3-N	*mgm. per cent NH_3-N*	*per cent*
0.9	1.0	−10.0
1.8	1.7	+5.6
2.5	2.6	−3.9
3.3	2.9	+13.8
5.5	5.2	+5.8
6.3	6.0	+5.0
6.8	6.7	+1.5
7.6	7.8	−2.6
8.5	8.2	+3.5
8.3	8.4	−1.2

the color remains uniformly distributed. The lowest determinable concentration was 0.5 mgm. per cent; with concentrations exceeding 10 mgm. per cent the standards were spaced at greater intervals.

Table 1 shows the results of 10 consecutive single analyses upon frog bladder urine to which, after preliminary treatment with permutit to remove ammonia, ammonium chloride was added in amounts which were unknown to the analyst. Analyses were not made in duplicate because of the unlikelihood that tubule fluid could be collected in sufficient volume to permit duplicates. It appears that there is no systematic error and that, though the percentage error may be considerable in the lower concentrations, the absolute error need not exceed 0.4 mgm. per cent and is usually much less than this.

Several additional facts support the propriety of using direct Nessleri-

zation in capillary tube colorimetry for the determination of ammonia in amphibian urine. Frog's urine itself appears colorless when examined in capillary tubes of 0.35 mm. diameter. Its creatinine concentration, as demonstrated by Höber (5) and confirmed by analyses in this laboratory, rarely exceeds 1.0 mgm. per cent; this amount is insufficient to influence the Nessler reaction (6). Frog's bladder urine, tested with Nessler's reagent after having been shaken with permutit, is colorless; hence we conclude that possible error arising from the presence of other color-producing substances than ammonia is insignificant.

The impression was obtained that, when volumes of fluid less than 0.2 c.mm. were tested, the color which developed was less intense than that obtained with larger volumes of fluid of the same concentration; for that reason, when only small amounts were available, the standard tubes were prepared with correspondingly small amounts. The traces of protein which tubule fluid sometimes contains (4) were found to be insufficient to affect color development.

Results. The results of 31 experiments in which fluid was collected from single nephrons of 22 frogs and 2 Necturi appear in figure 1. The site of each collection is indicated by the position which the dots bear relative to the diagrammatic nephron at the top of the figure. The concentration of ammonia nitrogen in each specimen is shown as percentage of that found in specimens of urine[3] formed during the fluid collections. None of the twenty specimens collected either from glomeruli, from any level of the proximal tubule, or from the proximal quarter of the distal tubule yielded measurable intensities of color and did not therefore contain ammonia nitrogen in excess of 0.5 mgm. per cent.[4] On the contrary (table 2), five of the eight collections from the last three quarters of the distal tubule and all of three collections from the collecting ducts con-

[3] Sixteen of these urine specimens were collected from the bladder and therefore represent the combined activity of both kidneys (range of ammonia nitrogen concentration 7.0 to 18.0 mgm. per cent, average 10.6); 15 were collected from the ureter of the same kidney in which a nephron was investigated (range of concentration 3.5–14.0 mgm. per cent, average 7.5).

[4] The threshold of the method, as has been stated, is in the neighborhood of this figure. Any or all of the specimens mentioned may therefore have contained an ammonia concentration of this order and each did, in fact, develop a barely detectable trace of color which is consistent with such a conclusion. The coloration was probably due to ammonia since a series of macro analyses performed with the method of Van Slyke and Hiller (7) by Miss Ethol Shiels indicated that the arterial blood of *R. catesbiana* contained on the average 0.54 mgm. per cent. It may therefore be tentatively concluded that the low concentrations of ammonia present in frog blood are filterable through the glomerular membrane. The inability of the ultramicro method to measure these concentrations quantitatively makes this conclusion a tentative one and forces us to refer to these fluids (in table 2 and fig. 1) as being free of ammonia.

tained definite concentrations of ammonia which, in the case of the latter group, approximated those shown by ureteral urine. No experiment is included in which there was any suspicion of contamination by fluid originating distally to the site of collection or in which the approximate identity of this site was not determined.

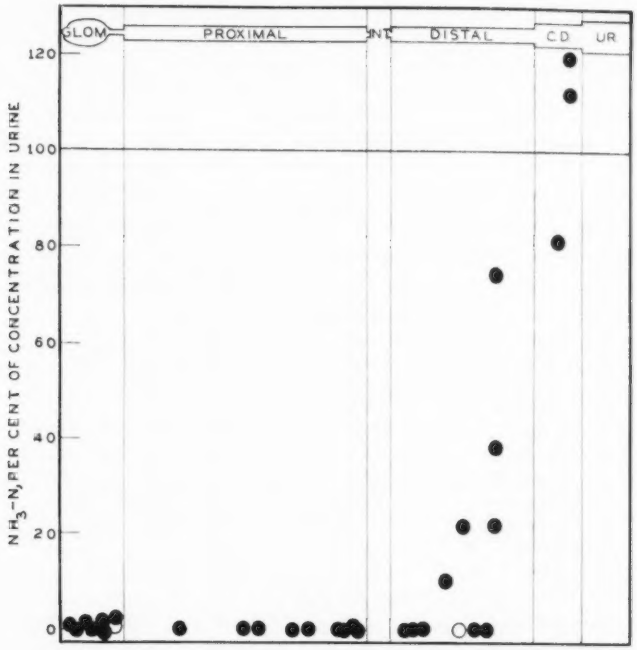

Fig. 1. Each solid dot represents an experiment upon a frog, each empty circle upon Necturus. The position of each symbol with reference to the diagrammatic nephron above indicates the portion of the unit from which the fluid was collected, its vertical position indicates the concentration of ammonia nitrogen it contained in terms of percentage of that contained by a urine specimen collected from ureter or bladder at the conclusion of the experiment. In computing these percentages a concentration of 0.5 mgm. per 100 cc. or less was regarded as 0.0 (see footnote 4). In the diagrammatic nephron: *Glom.* = glomerulus, *Int.* = intermediate tubule, *C.D.* = collecting duct, *Ur* = bladder or ureter.

These experiments prove that ammonia, other than the low concentrations which appear in blood plasma and are apparently present in glomerular fluid, does not appear within the nephron until the first quarter of the distal tubule has been passed; at about the point, that is, at which the change in pH has been shown to occur (8). Beyond this point and

throughout the remainder of the nephron there is a marked and progressive increase in ammonia concentration. This increase is far too great to be due to water reabsorption, the extent of which in the frog is accurately known (9) (10), nor do substances concentrated by this mechanism (11) show the sudden rise late in the distal tubule which ammonia presents (fig. 1). It cannot be due to secretion of ammonia from the peritubular blood supply for, in a series of 4 experiments, blood obtained from the posterior vena cava contained a higher concentration of ammonia than blood from an aortic arch[5] and (*vide infra*) ammonia continues to appear

TABLE 2

Collections of fluid from the distal tubules and collecting ducts of frogs and necturus

NUMBER	SPECIES	SITE OF COLLECTION*	RATE OF COLLECTION	AMMONIA NITROGEN IN		
				Tubule fluid	Ureteral urine	Bladder urine
			cu. mm. per hr.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
		Distal tubule				
1	*R. pipiens*	1/8	0.74	0.0†		18.0
2	*R. pipiens*	1/8	0.60	0.0		8.0
3	*R. pipiens*	1/4	0.51	0.0		12.0
4	*R. catesbiana*	1/3	0.20	1.4	14.0	
5	*R. pipiens*	1/2	0.19	4.0		18.0
6	*N. maculosus*	1/2	0.69	0.0	3.0	
7	*R. catesbiana*	2/3	0.24	0.0	3.5	
8	*R. catesbiana*	2/3	0.24	0.0	10.0	
9	*R. catesbiana*	3/4	0.53	2.0	9.0	
10	*R. pipiens*	3/4	0.82	1.5	4.0	
11	*R. pipiens*	3/4	0.34	3.0	4.0	
		Collecting duct				
12	*R. pipiens*	1/4	1.08	7.0	6.0	
13	*R. catesbiana*	1/2	3.00	3.5	4.0	
14	*R. pipiens*	3/4	4.50	6.0	5.0	

* The site of collection is expressed as the fraction of the segment of the nephron between its proximal end and the point of insertion of the pipette.

† 0.0 in this column means 0.5 mgm. per cent or less. See footnote 4.

in the urine when it is absent from the peritubular blood vessels. It must therefore be due to the formation of ammonia within this segment of the tubule lumen or in the cells which line it.

In an attempt to explore these latter alternatives we performed three experiments in which the kidneys of *R. catesbiana* were perfused with the fluid of Barkan, Broemser and Hahn (12); glycocoll was omitted and

[5] Average concentration of arterial blood was 0.54 and of venous blood 1.05 mgm. per cent ammonia nitrogen. The analyses were made by Miss Ethol Shiels employing the macro method of Van Slyke and Hiller (7).

the perfusion fluid therefore contained neither ammonia nor any known ammonia precursor. Ammonia continued to appear in the urine in rather high concentration (average 5 mgm. per cent) for one hour and did not completely disappear for two hours. This result is analogous to those described by Rehberg and Blem (13) during their investigation of the mechanism of urea excretion. Under these circumstances, then, the urinary ammonia must have been formed from some substance stored within the tubule cells. This substance is not ammonia itself, for we have not found appreciable amounts of ammonia in frog kidney extracts. It must therefore have been either urea or an amino acid. We were not able to distinguish between these alternatives by adding urea and glycocoll to the perfusion fluid for the excretion of ammonia, once it had ceased, could not be restored by either procedure. The existence of considerable deposits of urea within the frog kidney, demonstrated by Marshall and Crane (14), favors the suggestion that this substance is the precursor; since the deposits are said to lie chiefly within the cells of the proximal tubules and since ammonia does not appear within the tubule till well along in the distal segment, they could however only serve as the precursor if they entered the lumen as urea and were transformed into ammonia after they had progressed a considerable distance. There is no information as to the presence or absence of stores of amino acids within tubules of the frog's kidney.

One final point requires discussion: the discrepancy between the ammonia concentrations of fluid from the middle third of the distal tubule and of the urine, which is revealed by an examination of table 2, may suggest that ammonia formation also occurs in the collecting ducts, in the ureter, and even in the bladder. Our evidence opposes the latter two possibilities. Urine, allowed to stay in the bladder for two hours (with ureters ligated), did not develop any increase in ammonia concentration and, though urine from the lowest third of the ureter has frequently shown a higher ammonia concentration than that from the uppermost third, this relationship has been inconstant and appears more readily explained by differences in the degree to which various portions of a kidney concentrate ammonia under the conditions of our experiments; it is consistent with this suggestion that urine specimens, simultaneously collected from two kidneys, frequently show different ammonia concentrations. Our experiments do not exclude the collecting ducts as a possible additional site of ammonia formation but the differences in ammonia concentration between distal tubule fluid and urine would appear to be more logically explained by the three following circumstances. No fluid could be collected from the end of a distal tubule. Fluid was usually collected from tubules in which the flow was more rapid, and therefore the opportunity for ammonia concentration less, than in the majority of distal tubules. Comparison was

seldom made between fluid from a distal tubule and urine from the same portion of the kidney.

The demonstrated absence of detectable amounts of ammonia from tubule fluid until the final portions of the distal segment increases the validity of certain conclusions drawn from our former experiments with urea (15) in which the method of analysis failed to distinguish between urea and ammonia nitrogen.

SUMMARY

An ultramicro modification of the direct Nesslerization method for ammonia determinations is described which, by permitting quantitative analyses upon as little as 0.1 cu.mm. of fluid, has made possible the analysis of fluid collected from glomeruli and various portions of single renal tubules of the amphibian kidney.

Ammonia does not appear within the tubule lumen in appreciable amounts until the latter two-thirds of the distal tubule. Within this region, and possibly also in the early part of the collecting duct, the ammonia concentration gradually increases until it approximates that of ureteral urine. It is concluded that this portion of the nephron constitutes the site of ammonia formation. The source of this ammonia appears to be a precursor which is stored within the tubule cells, but the experiments did not resolve the question whether this substance is urea or an amino acid.

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VARIATION IN THE CONCENTRATION OF ACIDS OF THE GASTRIC CONTENT IN NORMAL SUBJECTS BEFORE AND FOLLOWING IMMERSION OF HAND AND ENTIRE BODY IN WATER AT VARIOUS TEMPERATURES

GRACE M. ROTH AND MILTON A. GABRIELSON

From the Section on Clinical Investigation, The Mayo Clinic, and the Mayo Foundation, Rochester, Minnesota

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In 1932 Horton and Brown, working with several patients who were hypersensitive to cold, produced, by immersing one hand of a patient in water at 10°C., a curve of gastric acidity similar to that produced by the administration of histamine. In 1937 one of us (Roth, 4) and Horton demonstrated that the rise in the concentration of gastric acidity produced by this exposure to cold approximated a subcutaneous injection of 0.4 mgm. of histamine.

A slight rise in the gastric acidity caused by the immersion of one hand in water at 10°C. has been shown in patients other than those with hypersensitiveness to cold. Therefore, the questions arose as to whether such a procedure would produce a rise of gastric acidity in normal subjects and further, whether immersion of the entire body up to the neck in water at various temperatures would produce a greater rise in the concentration of gastric acids.

PROCEDURE. The present work was carried out on five normal subjects. Each of the five subjects was subjected to a series of tests that included immersion of one hand in water at 10°C. (50°F.) and immersion of the whole body up to the neck in water ranging in temperature, with intervals of 5° from 65 to 108°F. (18.3 to 42.2°C.). Generally, several days elapsed between the testing of each subject at the different bath temperatures to avoid the development of a refractory period. Gastric analysis accompanied these procedures in the following manner: After fasting for twelve hours samples of gastric content were obtained by means of a tube of small bore and the gastric content was evacuated at each period. A control sample was aspirated fifteen minutes after introduction of the stomach tube. With the tube remaining in the stomach, the hand of the subject or the subject was immersed in the water bath for a period of fifteen minutes. At the end of this period, before the hand of the subject or the subject was removed from the bath, another sample was aspirated. After re-

moval of the hand or the subject from the water, two more samples were obtained, one after fifteen minutes, and the other after thirty minutes. Thus, in all, four samples were obtained. Each time the subject came out of the water he was allowed to lie on a bed and was covered with the same light blanket to eliminate the effect of different room temperatures.

During all the tests the subjects carefully expectorated saliva to avoid any neutralization of gastric acidity. For the same reason, any gastric specimens which were tinged with bile were discarded. Free and total gastric acidity were determined by titration, using Töpfer's reagent and phenolpthalein as indicators and were recorded in terms of units of tenth-normal sodium hydroxide.

Results. When one hand of each of the five subjects was immersed in water at 10°C. (50°F.) for fifteen minutes and the gastric acidity was determined as previously described, only a slight rise in the gastric acidity occurred, particularly fifteen to thirty minutes after removal of the hand from the water.

When the entire body of each subject was immersed up to the neck in water ranging in temperature, with intervals of 5° on each day, from 65 to 100°F. (18.3 to 37.8°C.), the greatest rise in the gastric acidity occurred between fifteen and thirty minutes after the subject was removed from the water (fig. 1). This was particularly true when the subject was immersed in baths at temperatures from 65 to 85°F. (18.3 to 29.4°C.). At this range of bath temperature the average rise in the gastric acidity was 28.2 units of tenth-normal sodium hydroxide for total acidity and 27.5 units for free hydrochloric acid with a range of increase from 16 to 54 units of tenth-normal sodium hydroxide for total acidity and from 12 to 54 units of tenth-normal sodium hydroxide for free hydrochloric acid. Fifteen minutes after removal of the subjects from baths ranging in temperature from 90 to 108°F. (32.2 to 42.2°C.) there was only a very slight rise in the gastric acidity in three subjects and in two instances there was a decrease.

There are various reports in the literature (2, 3) that deal with the effects of baths of different temperatures on the circulation. Although most workers agree that total metabolism is increased on exposure of the body to cold baths, there is considerable controversy as to whether the increase is produced solely through muscular contractions such as shivering or by other mechanisms. When these subjects were immersed and motionless in water at 65 to 75°F. (18.3 to 23.9°C.) there was considerable shivering at first. At the other temperatures there was no visible shivering.

Although the basal metabolic rates were unchanged before immersion and at the height of the increase in the gastric acidity, fifteen minutes after the removal of a subject from a bath at 75°F. (23.9°C.), the studies were not sufficiently comprehensive to justify the drawing of conclusions from them and further metabolic studies are now being carried out.

At a temperature of 75°F. (23.9°C.) there was no visible shivering and in order to determine whether this response of the gastric acidity of normal subjects fifteen minutes after immersion in water at 75°F. (23.9°C.) was a histamine or a histamine-like reaction, histaminase was employed. Commercially[1] available histaminase is obtained from the intestinal mucosa and the kidney, and the preparation for oral use is enteric coated. Consequently, it is probable that absorption takes place only after it reaches the intestine. Therefore, a special preparation was obtained which consisted of a fine white powder that could be suspended in normal saline and could be administered by means of a duodenal tube.

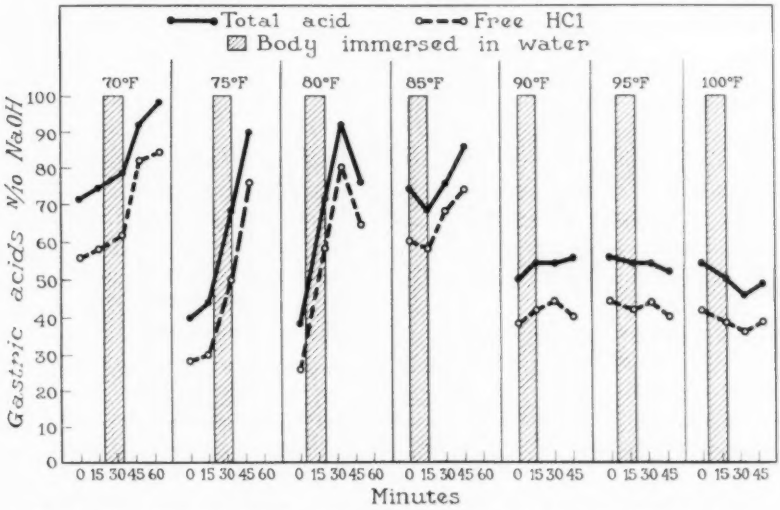

Fig. 1. The effects produced on the concentration of the gastric acidity by immersion of the body for fifteen minutes in baths at 70 to 100°F. (21.1 to 37.7°C.).

As in the previous observations, the subjects were immersed up to the neck in water at 75°F. (23.9°C.) for fifteen minutes and determinations of the gastric acidity were made before, during and for thirty minutes after the immersion in water. Several days later, histaminase was introduced into the duodenum of these same subjects twenty-five to thirty minutes before the immersion in water at the same temperature and for the same period of time. Following introduction of the histaminase into the duodenum and after subsequent washing of the histaminase into the duodenum with normal saline, the duodenal tube was withdrawn so that its tip would

[1] Histaminase was kindly supplied to us by the Medical Research Department of the Winthrop Chemical Company.

be in the stomach for aspiration of samples of gastric content. These samples were obtained at the same intervals employed in the previous procedure (fig. 2).

When 10 units of histaminase were introduced into the duodenum and samples of gastric content were obtained without immersion of the subject in water, there was little change in the concentration of gastric acidity (fig. 2*a*).

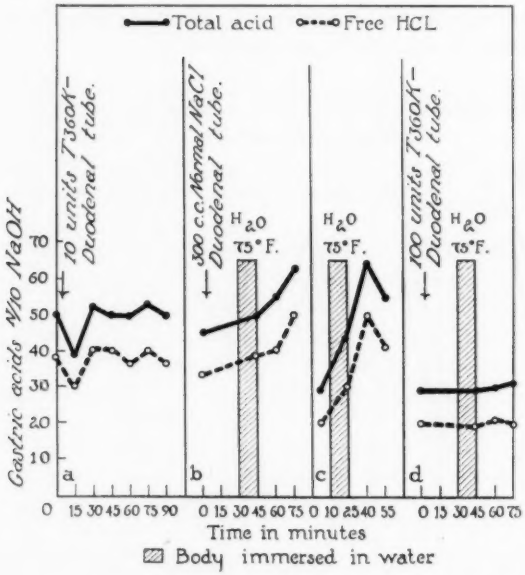

Fig. 2. Effects produced on the concentration of gastric acids; *a*, following the introduction of 10 units of histaminase into the duodenum; *b*, following the introduction of 300 cc. of physiologic saline into the duodenum thirty minutes before immersion of the subject in water at 75°F. (23.9°C.) for fifteen minutes; *c*, following immersion of the subject in a water bath at 75°F. (23.9°C.) for fifteen minutes (control observation); *d*, following the introduction of 100 units of histaminase into the duodenum thirty minutes before immersion of the subject in water at 75°F. (23.9°C.) for fifteen minutes. Note evidence of inhibitory effect of histaminase in *d*.

Since histaminase was introduced with normal saline, to rule out the factor of dilution, triple the amount of normal saline used to introduce histaminase was allowed to enter the duodenum thirty minutes before the subject was again immersed in water at 75°F. (23.9°C.). The concentration of the gastric acidity practically paralleled that present at the time of the control immersion of the subject (fig. 2*b* and *c*).

In each of these five subjects, when 100 units of histaminase were in-

troduced into the duodenum by means of the duodenal tube thirty minutes before the subject was immersed in water at 75°F. (23.9°C.), the usual increase in the concentration of gastric acidity following immersion of the subject in water at this temperature did not occur. Since this rise in the concentration of gastric acidity was inhibited by the introduction of histaminase, it would seem logical to conclude that the previous rise in gastric acidity most likely was produced by histamine or a histamine-like substance (fig. 2*d*).

SUMMARY

When the hand of each of five normal subjects was immersed in water at 10°C., there was only a slight increase in the gastric acidity during and fifteen minutes after the hand was removed from the water.

When these five normal subjects were immersed up to the neck in water ranging in temperature, with intervals of 5° on each day, from 65 to 85°F. (18.3 to 29.4°C.), a definite rise in the gastric acidity occurred particularly fifteen minutes after removal of the subject from the water.

Fifteen minutes after removal of the five normal subjects from water ranging in temperature, with intervals of 5° on each day from 90 to 108°F. (32.2 to 42.2°C.), only a very slight rise in the concentration of gastric acidity occurred in three subjects while there was a definite decrease in the gastric acidity in the other two subjects.

When histaminase was introduced into the duodenum by means of a duodenal tube thirty minutes before immersion of each of the five subjects in water at 75°F. (23.9°C.), the usual rise in the gastric acidity produced by water at this temperature was inhibited. This would seem to indicate that the rise in gastric acidity was probably due to the release of histamine or a histamine-like substance.

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SPECIES DIFFERENCES IN THE CHOLINERGIC ACTION OF ESTROGEN[1]

SAMUEL R. M. REYNOLDS AND FRANCES I. FOSTER

From the Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.

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Injection of estrogen into ovariectomized rabbits is followed within an hour by an increase in the free acetylcholine content of the uterus (Reynolds, 1939a; Reynolds and Foster, 1939). This is, accordingly, an example of the cholinergic action of estrogen in this species, and one which is demonstrable in the transplanted uterus, free of connection with the central nervous system (Reynolds, 1939a). In rabbits, there is also evidence that the sympathetic nerves to the uterus are cholinergic (Sherif, 1935; Reynolds, 1939b). Since, however, the sympathetic innervation of the uterus of the cat is adrenergic (Cannon and Bacq, 1931; Cannon and Rosenblueth, 1936), it was considered desirable to ascertain whether or not estrogen exerts a similar cholinergic action on the uterus of the cat. The following experiments record the results of such observations. In addition, experiments have been carried out on the uterus of the rat since there is evidence which purports to show by indirect means that in this species estrogen may not act in association with acetylcholine in the uterus (Holden, 1939; Astwood, 1940).

PROCEDURES. Ovariectomized animals were used. The operation was carried out from six to seventy days before the experiment, although eighteen to twenty-one days was most often the elapsed time between operation and experiment. In all, twenty-six cats and fifty-two rats were used in this work. Eleven of the cats were untreated controls, while fifteen were treated with estrogen. Twenty-one of the rats were used as untreated controls, thirty-one were treated with estrogen. Inasmuch as the rat uteri were very small, it was necessary to group them into lots of two to four uteri each. Seven of these were extracts from control rats, ten were from estrogen-injected ones.

The most frequently used estrogen was Amniotin (Squibb), as in the earlier experiments on rabbits, although Progynon-B (Schering) or Ben-Ovocylin (Ciba) was the hormone employed in a few experiments. There is no demonstrable difference between these preparations in their cholinergic action on the uterus of the rabbit (Reynolds and Foster, 1939).

[1] Aided by grants from the committee for Research in Problems of Sex, National Research Council, and the Josiah Macy, Jr. Foundation.

The amounts of hormone used were for the most part 10,000 rat units of Amniotin and 10,000 international units of the estradiol-benzoate products. Smaller quantities were employed in the early experiments until it was apparent that they were without effect upon the acetylcholine-content of the uterus. In the rabbit, it should be noted, 100 rat units of Amniotin per kilogram of bodyweight regularly increased the concentration of free acetylcholine in the uterus.

The method of extraction of the uterine tissues and of testing the extracts for an acetylcholine-like substance was exactly as in the earlier experiments (Reynolds, 1939a; Reynolds and Foster, 1939). It is the technic recommended by Chang and Gaddum (1933). The sensitivity of the frog's rectus abdominis muscle (the test-object employed) varied from 0.01 to 0.05 gamma of acetylcholine. Two experiments, not included in

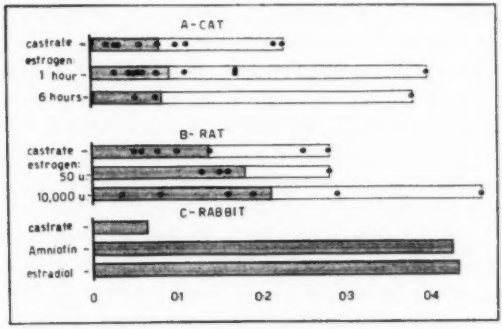

Fig. 1. Chart showing the absence of a cholinergic effect on the uterus of the cat and rat, contrasted with that seen in the rabbit. Dots, tests of individual extracts. Shaded areas, mean concentrations of an acetylcholine-like substance. Figures, gammas per gram of fresh tissue.

the groups above, were discarded because the least discernible response of the test-muscle was to 1.0 gamma of acetylcholine. The concentration of the extracts was one cubic centimeter for 0.3 to 1.5 grams of fresh tissue.

RESULTS. *In cats.* It will be seen in figure 1A that no difference was found in the acetylcholine-equivalent content of uteri from untreated and from estrogen-treated cats. This is indicated by the mean values obtained, and by the range of the individual points in each group of experiments. The mean concentration was found to be 0.08 to 0.09 gamma of an acetylcholine-like substance per gram of fresh tissue. In the untreated rabbit uterus, the concentration of such a substance is about the same (0.06–0.07 gamma per gram of fresh tissue). In contrast, the mean concentrations of acetylcholine in the uteri of estrogen-injected rabbits ranged from 0.42 to 0.79 gamma per gram of fresh tissue in different groups of experiments (Reynolds and Foster, 1939).

In order to exclude the likelihood that the time-course of a cholinergic effect in the cat differed appreciably from that found in the rabbit (where the maximum concentration of acetylcholine is observed one hour after injection of the hormone), five experiments were carried out with uteri taken six hours after injection of the estrogen. As will be seen in figure 1A, no increase in the amount of an active substance was found. One must therefore conclude that estrogen administered to cats, unlike the situation in rabbits, is without a cholinergic action upon the uterus.

In rats. In figure 1B, it will be seen that there is no apparent difference between the quantities of an acetylcholine-like substance in rat uteri before and after estrogen. The concentration of such a substance is about double that observed in uteri from castrated cats and from untreated, ovariectomized rabbits.

No attempt has been made in this work to correlate the values obtained with anything but the wet-weight of the organ. This was found to be adequate in the case of the rabbit. If the concentration of an acetylcholine-like substance in the uterus were correlated with the dry-weight, or perhaps with the nitrogen, or some other tissue constituent, significant differences in this respect possibly might be demonstrable. In view of the data available, however, we must conclude that a species difference clearly exists with regard to the cholinergic action of estrogen in the rabbit, on the one hand, and in the cat and rat, on the other.

SUMMARY

1. Estrogen has a cholinergic action on the uterus of the rabbit, independently of connections with the central nervous system.

2. In cats and rats, it is found that estrogen is devoid of a cholinergic action on the uterus.

3. Attention is called to the fact that in rabbits, both estrogen and the sympathetic nerves to the uterus are cholinergic, whereas in the cat, estrogen is without a cholinergic action on the uterus, and the sympathetic nerves to the uterus are adrenergic. The character of the uterine innervation in the rat has not been established.

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NATURAL ISOHEMAGGLUTINATION IN DOGS*

WILLIAM H. OLSON

From the Department of Gastro-Intestinal Research and the Samuel Deutsch Serum Center, Michael Reese Hospital, Chicago

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The phenomenon of isohemagglutination as described by Landsteiner (1) in 1900 in human beings was not as readily demonstrated in dogs. Von Dungern and Hirschfeld in 1910 (2) reported that distinct isoagglutination does not occur in the normal dog. They, however, demonstrated differences between erythrocytes of different dogs by the use of human typing serum, and thereby attempted to establish specific blood groups in dogs. The first to show that natural isohemagglutination occurred in dogs was Ottenberg, Friedman and Kaliski (3) in 1911, and they called it a weak phenomenon. Later studies by them (4) showed the dangers of incompatible transfusions between dogs. The most extensive work on natural isohemagglutination in dogs was done by McEnery, Ivy and Pechous (5) in 1924. They reported positive agglutinations in 533 instances out of 2,270 tests, but an attempt to establish groups was unsuccessful. On the other hand, Lauer (6) in 1930 obtained no positive agglutinations in over 200 tests. Recently Melnick, Burack and Cowgill (7) reported development of isohemagglutination in dogs following repeated injections of red blood cells of other dogs, upon which Melnick and Cowgill (8) in 1937 established the occurrence in dogs of two blood groups. The production of isohemagglutinins was confirmed by Wright in 1936 (9).[1]

Hemagglutination is a simple and clear cut phenomenon, and we were impressed by the fact that so much conflict of opinion existed concerning its occurrence in the dog. In numerous experiments on dogs in which blood, serum and plasma transfusions were employed (10) we discovered the essential factor in hemagglutinations in dogs: namely, that the isohemagglutinins are present in plasma but absent in the serum of dogs' blood. All the above investigators either stated or implied that blood serum was used in doing their agglutination tests.

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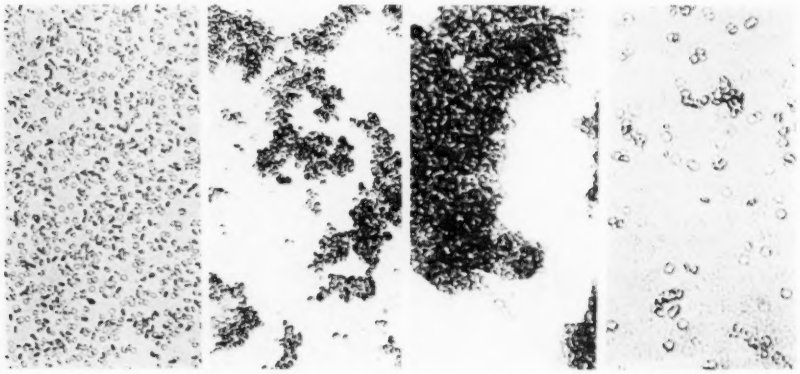
[1] A paper by B. Zvetkov "The Blood Groups of the Dog" Bjuleteni postijnoi Komisii Viccannja Krovjanich ugrupovan 1927, 1, 59-65, could not be obtained.

METHODS. Normal healthy dogs were picked at random; consequently a variety of animals as to breed, sex and age was observed. In each experiment, groups of nine to twelve animals were tested at one time against each other, i.e., 81 to 144 compatibility tests with plasma and double this number when parallel tests were made with serum. Care was exercised in obtaining blood for plasma by having the anticoagulating agent in the syringe. Various anticoagulating agents were employed and all seemed to be equally satisfactory. In most of this work sodium citrate has been employed. The erythrocytes rarely hemolyzed when placed in a mixture of sodium citrate and physiological saline solution. The Vincent open



A B C D

Fig. 1. (Micro-photographs—App. 1:100 Mag.) Typical reactions of dog erythrocytes when mixed with plasma using the Vincent open slide method.

Reading from left to right, A, B, C, D.

A. Negative reaction (−). No agglutination.

B. Positive reaction (+). Definite agglutination.

C. Strong positive reaction (++). Heavy agglutination.

D. Questionable reaction (∓). Little if any agglutination. Chiefly Rouleaux formation.

slide method was used in most of our work. Attempts had been made to employ Landsteiner's centrifuge method and when successful it checked the Vincent method. The difficulty in utilizing Landsteiner's procedure is the great tendency of dogs' erythrocytes to hemolyze. This hemolysis did not seem to have any relation to the phenomenon of isoagglutination. All tests were checked microscopically and were evaluated as follows: no agglutination, negative (−) (fig. 1 A), definite agglutination, one plus (+) (fig. 1 B) heavy agglutination, two plus (++) (fig. 1 C). Some agglutinations were doubtful (±) (fig. 1 D), and were counted as negative (v.i.).

RESULTS. Ninety-seven dogs were used and a total of 3355 compatibility tests were done. Breed, sex and age of the animals seemed to have no influence on the occurrence of natural isohemagglutination. In 2281 tests blood plasma was used as agglutinating fluid, with 974 or 42 per cent positive agglutinations. In 1074 tests blood serum was used as agglutinating fluid, with only 63 or 6 per cent positive agglutinations. Parallel tests on the plasmas corresponding to the serums resulted in 462 positive results, or 43 per cent. Table 1 illustrates a typical experiment on 12 dogs, using plasma as agglutinating fluid. Plasma and erythrocytes of each animal were tested against plasma and erythrocytes of all other animals in this group. The horizontal columns present the effects on the red cells of each dog of the plasma of all other dogs of the group. The vertical columns present the effects of the plasma of the individual dog

TABLE 1

Iso-agglutination in the plasma of a group of dogs

CELLS OF DOG NO.	PLASMA OF DOG NO.											
	1	2	3	4	5	6	7	8	9	10	11	12
1	−	++	−	++	±	++	−	+	++	+	+	+
2	−	−	−	−	−	−	−	−	±	±	−	−
3	−	++	−	+	−	+	++	++	+	±	++	−
4	−	±	−	−	−	±	±	−	−	+	−	−
5	−	++	−	++	−	++	+	+	+	+	+	−
6	−	±	−	±	−	−	−	−	−	+	−	+
7	−	+	+	+	−	++	−	+	+	++	−	−
8	−	−	−	−	−	±	+	−	−	−	−	−
9	−	+	−	+	−	+	+	−	−	++	−	±
10	−	++	±	++	−	±	+	+	+	−	+	±
11	−	+	−	+	±	+	−	+	−	+	−	+
12	−	++	−	++	−	++	+	+	+	+	+	−

TABLE 2

Iso-agglutination in the serum of a group of dogs

CELLS OF DOG NO.	SERUM OF DOG NO.											
	1	2	3	4	5	6	7	8	9	10	11	12
1	−	−	−	±	−	±	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	+	−	±	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	−
7	−	−	−	−	−	−	−	−	−	−	−	−
8	−	−	−	−	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−	−	−	−
10	−	−	−	±	−	−	−	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−
12	−	−	−	+	−	−	−	−	−	−	−	−

on the erythrocytes of all other dogs of the group. Fifty-nine definite agglutinations were observed, an incidence of 41 per cent. Table 2 illustrates a typical experiment using serum as the agglutinating fluid performed on the same dogs and at the same time as the plasma agglutinations in table 1. Only two definite agglutinations were observed, an incidence of 1.4 per cent.

Repeated tests were done on the same dog using plasma as the agglutinating fluid in order to learn whether the phenomenon of isoagglutination was constant or only transient in the individual dog. Only questionable changes occurred in many repeated tests on the same animals, and these appeared to be associated with the health of the animal. Canine distemper and its complications definitely affected isoagglutination. On several occasions distemper caused pseudo- or auto-agglutination, between erythro-

cytes and plasma or serum obtained from the same dog. This latter phenomenon was not observed in healthy animals. Seven healthy dogs were observed over a six months' period and identical results were obtained in frequent compatibility tests.

The incidence of positive agglutinations with blood serum was much higher in the early stages of this study. This was due to the fact that at that time the serum was improperly prepared, i.e., the fibrinogen was not completely removed. When freshly clotted blood was centrifuged at high speed, the resultant serum was not completely free of fibrinogen, and resulted in 45 positive agglutinations in 308 tests, an incidence of 15 per cent. This incidence was reduced markedly by allowing the blood to stand for 12 to 16 hours during which time more fibrin precipitated out; at this time only 10 agglutinations occurred in 624 tests, an incidence of 1.6 per cent as compared to the 15 per cent agglutinations from serum which still contained fibrinogen. In a further effort to completely remove all fibrinogen, serum obtained from recalcified citrated plasma was used, with not a single instance of agglutination in 243 consecutive tests. This confirms Lauer's work (6) previously mentioned. McEnery's (5) 23 per cent incidence of agglutination was probably obtained with serum containing fibrinogen. This is supported by McEnery's remark that on standing for 1 week, the serum lost its agglutinating power. This loss of agglutinating power is obviously due to the fact, frequently observed in our studies, that on standing fibrin will precipitate out from serum prepared quickly from freshly clotted blood. Even citrated plasma, on standing, will lose its fibrinogen and, with it, the property of agglutinating red blood cells. Table 3 illustrates a typical experiment showing the rôle of fibrinogen in iso-agglutination.

The agglutinated erythrocytes appear to be more fragile than those remaining free. When partial hemolysis[2] has occurred, the remaining intact red blood cells show a greatly diminished tendency to agglutinate. In the experiment presented in table 4, 38 positive and 5 questionable results in 81 tests on non-hemolyzed blood were reduced to 3 positive and 11 questionable results, by hemolyzing 75 to 80 per cent of the cells; positive agglutination of such cells appeared only on standing for 15 minutes. Therefore partially hemolyzed blood should not be used for agglutination tests if accurate results are desired. Great care must be exercised when working with dogs' blood for their erythrocytes are more fragile than those of man. This readiness to hemolyze has been observed by other investigators (3).

In analyzing our work, we noted that the incidence of isohemagglutination was similar in certain animals thus indicating the possibility that blood groups may exist in dogs. In order to classify different animals as

[2] Hypotonic saline solution was used.

belonging to one group, three conditions should be met. Their blood should be mutually compatible; their erythrocytes should react similarly

TABLE 3

Rôle of fibrinogen in iso-agglutination. A. Citrated plasma

CELLS OF DOG NO.	PLASMA OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	+	−	+	+	−	+	++
14	+	−	±	−	+	+	−	+	++
15	+	−	−	−	+	+	−	+	+
16	+	−	−	−	+	+	+	+	+
6	+	+	−	−	−	−	−	+	−
17	±	−	+	−	−	−	−	−	−
18	+	−	±	−	+	+	−	+	±
19	±	−	+	−	±	+	−	−	+
20	±	−	−	−	−	−	−	−	−

Rôle of fibrinogen in iso-agglutination. B. Serum from clotted blood

CELLS OF DOG NO.	SERUM OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	±	−	±	−	−	−	+
14	−	−	−	−	−	−	−	±	+
15	+	−	−	−	−	−	−	−	±
16	−	−	−	−	−	+	−	±	−
6	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−
18	−	−	−	−	+	−	−	−	−
19	−	−	−	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−	−

Rôle of fibrinogen in iso-agglutination. C. Serum from recalcified plasma

CELLS OF DOG NO.	SERUM OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	−	−	−	−	−	−	−
14	−	−	−	−	−	−	−	−	−
15	−	−	−	−	−	−	−	−	−
16	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−
19	−	−	−	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−	−

TABLE 4

Controls for iso-agglutination in non-hemolyzed blood

CELLS OF DOG NO.	PLASMA OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	+	−	+	+	−	+	++
14	±	−	±	±	+	+	−	+	++
15	+	−	−	−	+	+	−	+	+
16	+	+	+	−	++	+	+	+	++
6	+	−	+	−	−	−	+	+	−
17	+	−	−	−	−	−	−	+	−
18	+	−	±	−	+	+	−	±	+
19	+	−	+	−	+	+	+	−	+
20	+	−	−	−	−	−	−	−	−

Iso-agglutination in partly hemolyzed blood (75 to 80% hemolysis)

CELLS OF DOG NO.	PLASMA OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	±	−	±	−	−	−	±
14	−	−	−	−	−	−	−	−	+
15	−	−	−	−	−	−	−	−	±
16	+	−	±	−	±	−	±	±	+
6	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−
19	±	−	−	−	−	−	−	−	±
20	±	−	−	−	−	−	−	−	−

with other plasmas, and their plasma or serum should react alike with other red cells. Table 1 shows that the bloods of dogs 1, 3 and 5 reacted

in identical fashion. In table 5 the results from table 1 have been regrouped. The blood of each dog in group I fulfills the three criteria mentioned above, i.e., they may belong to one blood group. This group is comparable to the AB type in humans using Landsteiner's nomenclature, because the plasma agglutinates no other cells and the cells are agglutinated by all plasmas of other possible groups. The blood from dogs 2, 4, 6 and 8 reacted entirely different from those in group I. They are compatible with each other and their red cells and plasma react similarly. This group is comparable to the O type in the human. The remaining dogs seem to belong to an intermediate group consisting of 2 or more groups, which may be separated in the future.

TABLE 5

Possible grouping of dogs

GROUP NO.	CELLS OF DOG NO.	PLASMA OF DOG NO.											
		Group I			Group II				Intermediate group				
		1	3	5	2	4	6	8	7	9	10	11	12
I	1	−	−	±	++	++	++	+	−	++	+	+	+
	3	−	−	−	++	+	+	++	+	+	±	++	−
	5	−	−	−	++	++	++	+	+	+	+	+	−
II	2	−	−	−	−	−	−	−	−	±	±	−	−
	4	−	−	−	±	−	−	−	−	−	+	−	−
	6	−	−	−	+	−	−	−	−	−	+	−	+
	8	−	−	−	−	−	−	−	+	−	−	−	−
Intermediate	7	−	+	−	+	+	++	+	−	+	++	−	−
	9	−	−	−	+	+	+	−	+	−	+	−	±
	10	−	−	−	++	++	±	+	+	+	−	+	±
	11	−	−	−	+	+	+	+	−	−	+	−	+
	12	−	−	−	++	++	++	+	+	+	±	+	−

The effect of transfusion of blood whose incompatibility had been demonstrated in vitro by iso-agglutination with plasma, was observed in several experiments. Different types of reactions were noted. In the most severe type the animal went into a profound shock before the infusion was completed and died following convulsions and paralysis four hours after the transfusion. Another type of reaction was less severe, and would have passed unnoticed if blood pressure had not been taken throughout and following the period of infusion. It consisted of a transient drop in blood pressure of 50 to 80 mm. Hg from which the animal recovered within one hour after transfusion. In other cases although there was no effect on blood pressure other reactions were noted, such as anuria and hemoglobinuria which sometimes cleared up after several days. There is no

doubt, however, that in many cases transfusion of incompatible blood will produce no symptoms in dogs. This is due to the weakness of the natural isohemagglutination in dogs. The titer of the agglutinins in dog's blood is approximately 1:4, whereas in man the titer is much higher.

SUMMARY

1. The blood plasma of dogs contains natural isohemagglutinins which are absent in the blood serum.

2. Complete removal of fibrinogen from the plasma removes the property of hemagglutination.

3. Those erythrocytes in the dog that are agglutinable are the most fragile cells.

4. It is believed that dogs may be divided into blood groups.

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THE MOBILIZATION OF VITAMIN A BY THE SYMPATHICO-ADRENAL SYSTEM[1]

GENEVIEVE YOUNG AND GEORGE WALD

From the Biological Laboratories of Harvard University, Cambridge, Mass.

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Drummond and MacWalter reported in 1934 that the removal of a single lobe of liver in the rat results in a large decrease in the vitamin A and carotene content of the remainder of the organ. These workers had attempted to follow the conversion of injected carotene to vitamin A *in vivo*, using the extirpated lobe as a control. The changes induced by the operation itself balked this procedure.

In the course of a study of carotene metabolism in rabbits we have performed similar experiments. In our initial work a lobe of liver was tied off and removed under ethyl urethane anesthesia. Its carotene and vitamin A concentrations were determined immediately by methods described below. Three to five hours following this operation the animal was killed with a blow on the head, and the remainder of the liver was quickly removed and similarly analyzed. The vitamin A and carotene content of the second portion of liver had invariably decreased, the vitamin A by an average of 23 per cent, the carotene by about 50 per cent (table 1).

There is no obvious reason why any part of this procedure should have promoted the actual destruction of carotene and vitamin A. It is more probable that the rapid removal of these substances from the liver is due to their transport to other tissues. This consideration led us to repeat the experiments, adding simultaneous determinations of blood vitamin A. Blood carotene is so low in these animals that we were unable to estimate it reliably.

The animals first were fasted for 24 hours in order to eliminate variations in blood vitamin A due to absorption from the intestine. Just before the removal of a lobe of liver, 10 ml. of blood were drawn from an ear vein under anesthesia. Another 10 ml. of blood were taken from the same animal just before it was killed to obtain the remaining liver tissue. The vitamin A content of each sample was determined at once (v. below). Simultaneous with its fall in the liver, the vitamin A concentration of the blood was found to rise 3 to 7.5 (average 6.4) times (table 1). Following

[1] This research was supported in part by a grant to one of us (G. W.) from the Josiah Macy, Jr. Foundation.

the extirpation of a lobe of liver, therefore, the remainder of the organ releases vitamin A into the blood stream.

The mobilization of sugar from its reserves in the liver under similar circumstances is known to be associated with stimulation of the sympathico-adrenal system (Cannon, 1928). It may also be evoked by direct stimulation of the liver through the splanchnic (sympathetic) nerve, or indirectly by stimulation of the adrenal glands, or by the injection of adrenaline. Certain plasma proteins—e.g., fibrinogen—appear to be mobilized from stores in the liver by similar means (Foster and Whipple, 1922; Stübel, 1920; Riecker and Winters, 1931). In an attempt to define the stimuli for the discharge of vitamin A more closely, therefore, we turned to direct stimulation of the sympathico-adrenal system.

TABLE 1

Vitamin A and carotene concentrations of liver and whole blood just before and 3 to 5 hours after the removal of a lobe of liver

Concentrations in micrograms (γ) per gram of fresh liver or per ml. of blood

RABBIT	LIVER				BLOOD VITAMIN A	
	Vitamin A		Carotene			
	Before	After	Before	After	Before	After
1	42.9	29.7	0.036	0.023		
2	34.1	31.0	0.154	0.098	0.148	0.444
3	98.8	76.2	0.105	0.049	0.296	2.22
4	42.9	32.7	0.148	0.040	0.203	1.48
Averages.....	54.7	42.4	0.111	0.053	0.216	1.38

Electrical stimulation of the greater splanchnic nerve in the anesthetized rabbit with liver intact caused an increase in blood vitamin A of about 85 per cent (table 2).[2] Similar stimulation of the middle cervical sympathetic ganglion, which innervates primarily structures in the head, produced no appreciable effect on blood vitamin A (table 2). The mobilization of vitamin A through nerve action appears to depend upon direct stimulation of the abdominal viscera, probably primarily of the liver itself, and, as the following experiments show, of the adrenals.

The vitamin A content of the blood was found to be unchanged just before and 30 minutes after the intravenous injection of 1 ml. of normal saline solution. When in an otherwise identical procedure in the same rabbit the saline solution was replaced with 1 ml. of 1:1000 adrenaline,

[2] The nerve was stimulated with rapidly interrupted induction shocks set just too weak to cause contraction in nearby muscles. Ten seconds of stimulation were alternated with 10 seconds of rest for a total stimulation period of 2 minutes.

the blood vitamin A rose 75 per cent (table 2, rabbit 6). In rabbit 5 the injection of adrenaline yielded a much greater rise in blood vitamin A (about 170 per cent) than had stimulation of the splanchnic nerve. In general, following injection of 1 ml. of 1:1000 adrenaline, the blood vitamin A approximately doubled within 30 minutes, then slowly declined. In one animal (no. 6) tested four hours after the injection it still had not returned to the basal level.

Vitamin A is mobilized from its reserves in the liver, therefore, by a pattern of stimuli comparable with those which mobilize sugar and apparently certain plasma proteins.[3] All these phenomena form part of the sympathico-adrenal syndrome, most components of which play clearly recognizable rôles in the maintenance of the organism (Cannon, 1939).

TABLE 2

Vitamin A concentrations in micrograms per ml. of whole blood, just before and at various times after the procedures indicated

TREATMENT	RABBIT	BLOOD VITAMIN A				
		Before treatment	After treatment (minutes)			
			10	30	90	240
Stimulation of splanchnic nerve	5	0.252	0.466			
Stimulation of cervical ganglion	6	0.166	0.163			
Injection of 1 ml. normal saline	6	0.174		0.178		
Injection of 1 ml. 1:1000 adrenaline	6	0.176		0.307		0.229
Injection of 1 ml. 1:1000 adrenaline	5	0.248		0.666		
Injection of 1 ml. 1:1000 adrenaline	7	0.277		0.540		
Injection of 1 ml. 1:1000 adrenaline	7	0.292		0.555		
Injection of 1 ml. 1:1000 adrenaline	8	0.207	0.359	0.344	0.270	
Injection of 1 ml. 1:1000 adrenaline	9	0.174	0.398			

Some significant rôle might possibly be assigned to the mobilization of vitamin A also, if more were known of its general cellular functions. As yet we understand reasonably clearly only the participation of this vitamin in the visual cycle of the rods, and associated with this the rise of night-blindness in vitamin A deficiency (Wald, Jeghers and Arminio, 1938). The curious possibility exists that the mobilization of vitamin A

[3] Clausen *et al.* (1940) have reported the mobilization of vitamin A in the blood of dogs after feeding or injection of ethyl alcohol. The average rise in serum vitamin A 1½ hours after the introduction of alcohol by stomach tube was 62 per cent; but the vitamin A continued to rise beyond this value, in some cases for as long as 48 hours. Some evidence is presented of a direct effect of alcohol on the liver; it is suggested also that traumatic effects on other tissues may contribute to the result. The relation of these to the present observations is still obscure.

might temporarily alleviate dietary night-blindness, and this if true should lend it something of the emergency character of other sympathico-adrenal responses.

METHODS. *Liver carotene and vitamin A.* Fresh liver was ground thoroughly in a mortar with an equal weight of anhydrous sodium sulphate. The mixture was dried under low pressure at about 40°C. for 2 hours. It was ground to a fine powder and Soxhletted with low boiling benzine (petroleum ether, boiling range 20°–40°C.); matters were so arranged that the solvent passed through the material about 24 times. The benzine was distilled off under reduced pressure. The oily residue was taken up in 5 ml. of 6 per cent KOH in methanol, and saponified at 55°C. for 45 minutes under a stream of nitrogen. The saponification mixture was cooled, diluted with water to a methanol content of 65 per cent, and was shaken with 5 successive portions of benzine. The combined extracts were reduced to a volume of about 10 ml. and were partitioned in the following way: the benzine solution was shaken twice with equal volumes of 90 per cent methanol, then the combined methanol solutions were re-shaken once with fresh benzine. The benzine fraction was washed with water, evaporated to dryness, and taken up in chloroform for carotene determination. Several solutions prepared in this way have been checked spectrophotometrically; they possess the familiar absorption spectrum of carotene.

The methanol fraction was diluted with water to a methanol content of 65 per cent, and was extracted 5 times with benzine. The benzine was distilled off under suction, and the residue taken up in chloroform and combined with the benzine fraction (above) for the vitamin A determination. On mixing these solutions with antimony chloride reagent a clear blue color results, due to the single sharp band maximal at 620 mμ which is specific for vitamin A.

Blood vitamin A. For each test approximately 10 ml. of blood were drawn from an ear vein into a beaker containing a few crystals of potassium oxalate. To the whole blood was added three times its volume of 98 per cent methanol, and the mixture was centrifuged. The supernatant solution was decanted, brought to a methanol content of 65 per cent, and was extracted three times by shaking with benzine. The precipitate was shaken separately with 5 successive portions of benzine. All extracts were combined, washed with water, and the benzine distilled off under suction. The residue was taken up in chloroform for vitamin A determination.

Vitamin A and carotene concentrations. These were determined with the Zeiss Pulfrich Photometer, the use of which for such measurements was described several years ago by Wald (1935–36).

Vitamin A was determined by the following procedure. Three-tenths

milliliter of the chloroform solution to be tested was pipetted into a 10 mm. absorption cell and placed in position in the instrument. To this, 3.2 ml. of saturated antimony trichloride solution in chloroform were added, and the transmission of the resultant blue mixture was read within 12 to 15 seconds, through the S61 filter. This filter transmits a narrow band of wavelengths which coincides closely with the antimony chloride band of vitamin A. From the transmission, I/I_0, in which I_0 is the incident and I the transmitted intensity, the optical density or extinction, log (I_0/I) is computed. This is directly proportional to the vitamin A concentration, in accordance with Beer's Law. The optical density multiplied by 37 yields directly the concentration of vitamin A in micrograms per milliliter of the original solution, before the addition of the antimony chloride reagent.

The factor 37 was obtained through measurement by the present method of a large number of vitamin A preparations whose direct absorptions at 328 mμ were already known (Wald, unpublished observations). This factor was computed on the basis that the density at 328 mμ of a 1 per cent chloroform solution of pure vitamin A, 10 mm. in depth, is 1600.

Carotene concentrations were determined directly by measuring the densities of chloroform solutions through the S50 filter of the instrument. These solutions also obey Beer's Law over the range of the measurements. The method was calibrated with standard chloroform solutions of crystalline carotene (about 90 per cent β and 10 per cent α isomer). It was found that the density of a 10 mm. layer multiplied by 7.0 yields the concentration of carotene in micrograms per milliliter.

SUMMARY

The extirpation of a lobe of liver in the rabbit results in decreases of about 25 per cent in the vitamin A content and 50 per cent in the carotene content of the remainder of the organ. Simultaneously the blood vitamin A rises 3 to 7.5 times.

Electrical stimulation of the splanchnic nerve also results in a large increase in blood vitamin A. Similar stimulation of the cervical sympathetics is without effect.

Following the intravenous injection of 1 ml. of 1:1000 adrenaline, blood vitamin A is approximately doubled within 30 minutes, then slowly falls.

Vitamin A is mobilized from its reserves in the liver, therefore, by sympathico-adrenal stimulation comparable with that which mobilizes sugar and certain plasma proteins.

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OBSERVATIONS ON DENERVATED MUSCLE IN RELATION TO MYOTONIA

ABE RAVIN[1]

From the Department of Physiology in the Harvard Medical School

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Myotonia is a muscular defect, practically always inherited, in which stimulated muscles remain contracted for an abnormally long time after the stimulus has ceased. Striking similarities in the properties of denervated and myotonic muscles suggested that an investigation of denervated muscle might throw some light on the nature of myotonia.

METHOD. Cats under dial (Ciba, 0.75 cc. per kgm., intraperitonally) were used. The quadriceps muscle was examined in most experiments; a few experiments were performed on the gastrocnemius-soleus. The femoral, or sciatic, nerve was sectioned aseptically 7 to 24 days before the experiment. The femur or tibia was firmly fixed by means of drills, and the muscle tendons attached to the short end of a writing lever pulling against rubber bands. For direct stimulation steel needles were inserted into the body and tendon of the muscles. Condenser discharges were used most commonly for stimulation; in a few experiments a multivibrator circuit was employed.

As a rule drugs were injected into the aorta by retrograde cannulation of the inferior mesenteric artery. The adrenals were routinely ligated.

For recording the action currents of the spontaneous fibrillary activity, needles were inserted into the muscle and the potentials, after amplification, were observed and photographed in a cathode-ray oscillograph.

RESULTS. A. *Prolongation of the first contraction after a period of rest.* 1. Description. If a muscle which has been denervated for a week or more is stimulated electrically with a single shock, such as from a condenser, the first contraction after a period of rest almost always shows a more prolonged relaxation than succeeding contractions. An initial phase of fairly rapid relaxation changes to a phase of slow relaxation which may last 5 to 15 seconds or longer (fig. 1). In the more marked instances the rapid phase may be short, and most of relaxation occurs slowly. The change from the rapid to the slow phase is frequently abrupt (fig. 1). The extent of the prolongation of the contraction varies from animal to animal; the

[1] Medical Fellow of the National Research Council.

factors causing this variation have not been determined, but they do not appear to be related to the length of denervation.

If the muscle is stimulated again immediately after it has relaxed the second contraction shows little or none of the slow phase of relaxation. The same is true of succeeding contractions. If, however, the muscle is permitted to rest for 2 or 3 minutes and then stimulated, the first contraction again shows the phase of slow relaxation, equal to its original value; the prolonged contraction, therefore, can be quite accurately duplicated at 3-minute intervals. In some animals the phase of slow relaxation is marked at the beginning of an experiment but decreases gradually in spite of long rest periods.

If the muscle is stimulated repeatedly before the slow phase of relaxation is completed, the resulting contractions are superimposed on the slow

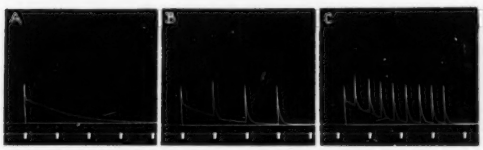


Fig. 1

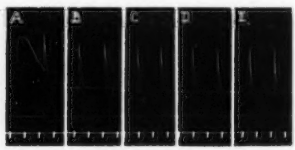


Fig. 2

Fig. 1. Prolongation of first contraction after a period of rest. Quadriceps denervated 16 days. Condenser shocks. A, B and C were taken at 3-minute intervals. Time in 5-second intervals. In A, the muscle was stimulated only once; in B, 4 times at 5-second intervals; and in C, 9 times at 2-second intervals.

Fig. 2. Increase in rapidity of relaxation on continued stimulation. Quadriceps denervated 7 days. Shocks from multivibrator circuit at about 9-second intervals. Time: 5-second intervals. A, first and second responses. B, 15th and 16th responses. C, 30 and 31st responses. D, 45th and 46th responses. E, 65th and 66th responses.

relaxation with little change in the elevated baseline (fig. 1). Occasionally the second contraction appears to cause a little drop in the baseline but none of the contractions raises it.

Although the markedly prolonged relaxation occurs only with the first contraction, relaxation of the second and succeeding contractions is slower than normal. Continued stimulation at 3- to 5-second intervals often produces a gradual increase in the rate of relaxation (fig. 2).

Rosenblueth and Luco (1937) noted that the prolonged contraction-remainders following electrical stimulation of denervated muscles were associated with action potentials. This would indicate that they were propagated contractions rather than contractures. The possibility, however, that some component of the contraction-remainder is due to contracture is not ruled out.

2. Action of adrenaline. Since stimulations at 3-minute intervals

elicit the slow phase of relaxation to about the same degree (fig. 1), the effects of drugs and various procedures on this phase can readily be determined. If the quadriceps muscle is tested at 3-minute intervals and the animal is then given an injection of adrenaline, subsequent tests for a period of 15 or more minutes show a marked increase in the speed of relaxation. This result was always obtained in the quadriceps muscle (fig. 3). Slowness of relaxation returns for the most part in 15 to 20 minutes and at

Fig. 3

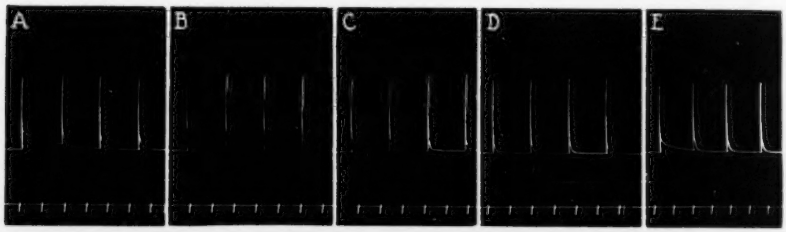


Fig. 4

Fig. 3. Effect of adrenaline on the prolonged first contraction. Quadriceps, denervated 24 days. Adrenals ligated. Shocks from multivibrator circuit at approximately 9-second intervals. Series of 4 contractions were made at 3-minute intervals. Time: 5-second intervals. A and B, 2 series made before giving adrenaline. C, 30 seconds after giving 25 γ of adrenaline intra-arterially. D, 3.5 minutes after C. E, 22 minutes after D.

Fig. 4. Effect of circulatory arrest compared with the effect of adrenaline on the prolonged first contraction. Quadriceps, denervated 14 days. Adrenals ligated. Condenser shocks. Series of 4 contractions (at 5-second intervals) were made at 3-minute intervals. Time: 5-second intervals. The circulation to the muscle was stopped by clamping the abdominal aorta from just before series A until just after series B. Series C was recorded after restoring the circulation and just before giving adrenaline; series D, 2 minutes after injecting 25 γ of adrenaline into the jugular vein.

times there is a slight but definite accentuation of the slow relaxation after 25 to 30 minutes. These effects can be seen following the injection of 25 γ of adrenaline into the jugular vein.

The possibility suggests itself that the action of adrenaline is on the blood supply rather than directly on the muscle. It can be shown, however, that whereas an injection of adrenaline produces a marked effect within 2 minutes, stopping the circulation to the muscle for 3 minutes de-

creases the length of the contraction little if at all (fig. 4). The conclusion that adrenaline acts mainly or entirely on the muscle seems justified.

The effect of adrenaline on the gastrocnemius-soleus muscles is quantitatively different from that on the quadriceps. An injection of adrenaline is followed by an increased speed of relaxation occurring within 2 to 4 minutes after the injection, but the improvement is much less than in the quadriceps. A brief period of improvement is followed by a longer period during which the slow phase of relaxation gradually increases, reaches a maximum in 15 to 19 minutes, and then gradually returns to its original extent (fig. 5). In this case, then, an initial decrease in the relaxation rate is of small degree and short duration, whereas the secondary increase in the relaxation rate is marked and prolonged.

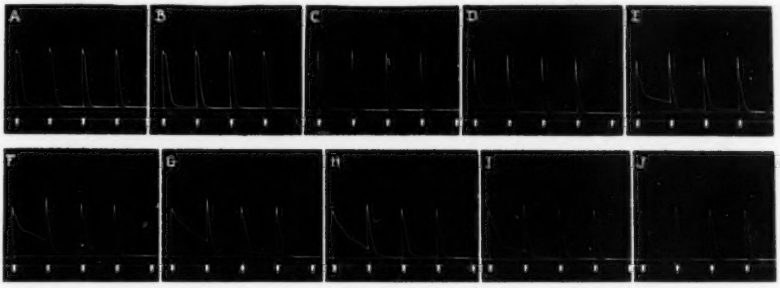


Fig. 5. Effect of adrenaline on the prolonged first contraction. Gastrocnemius-soleus, denervated 15 days. Condenser shocks. Adrenals ligated. Series of 4 contractions made at 3-minute intervals. Time: 5-second intervals. A and B, taken before giving adrenaline. One minute before C, 25 γ of adrenaline injected into aorta. C to J, show successive series at 3-minute intervals.

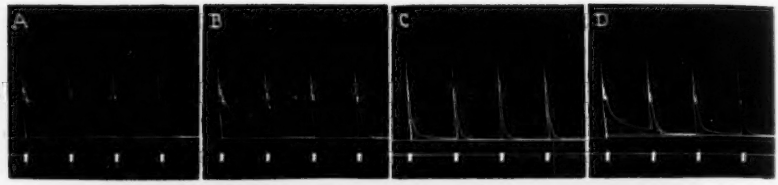
3. Action of potassium. Potassium chloride (5–30 mgm.) injected intra-arterially 20 to 30 seconds before a test produces a marked decrease in the duration of the contractions (fig. 6). The effect is evident for only 2 or 3 tests (at 3-min. intervals) and occurs in both quadriceps and gastrocnemius-soleus muscles with doses of wide range. No secondary phase of accentuation is seen.

4. Action of quinine. Quinine markedly decreases the duration of the contractions; both the rapid and slow phases of relaxation are involved (figs. 7 and 8). An increase in extent of contraction also is usually evident (fig. 7).

5. Action of calcium. Calcium chloride (15 mgm.) injected intra-arterially decreases the duration of the contractions (fig. 9). It produces also a decrease in the extent of contraction. As the effect wears off the duration and extent of the contractions increase.

6. Action of eserine and prostigmin. No clear-cut effect of eserine or

Fig. 6



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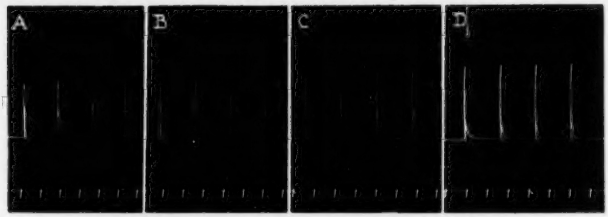

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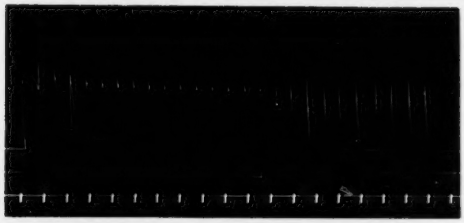

Fig. 9

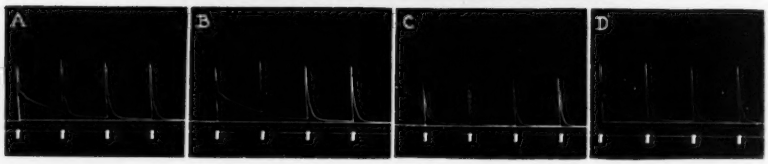

Fig. 6. Effect of potassium chloride on the prolonged contractions. Quadriceps, denervated 16 days. Adrenals ligated. Condenser shocks. Series of 4 contractions were taken at 3-minute intervals. Time: 5-second intervals. A and B, before giving potassium chloride. C, 30 seconds after injecting 15 mgm. of potassium chloride into aorta. D, 3 minutes after C.

Fig. 7. Effect of quinine on the prolonged contractions. Quadriceps, denervated 24 days. Adrenals ligated. Stimulation by multivibrator circuit at approximately 9-second intervals. Series of 4 contractions at 3-minute intervals. Time: 5-second intervals. A and B, before giving quinine. C, 2.5 minutes after injection of quinine hydrochloride (15 mgm.) into aorta. D, 8.5 minutes after C.

Fig. 8. Effect of quinine on the contractions of denervated muscle. Quadriceps, denervated 7 days. Stimulation by multivibrator circuit at approximately 3-second intervals. Time: 5-second intervals. At signal 15 mgm. of quinine hydrochloride injected into aorta.

Fig. 9. Effect of calcium chloride on the prolonged first contraction. Quadriceps, denervated 16 days. Condenser shocks. Series of 4 contractions at 3-minute intervals. Time: 5-second intervals. A and B, before giving calcium. C, 0.5 minute after injecting calcium chloride (15 mgm.) into aorta. D, 18 minutes after C.

prostigmin could be shown. In 2 experiments a questionable increase in duration of relaxation was seen. With prostigmin small doses produced no effect; larger doses produced a shortening of the muscle which interfered with further tests.

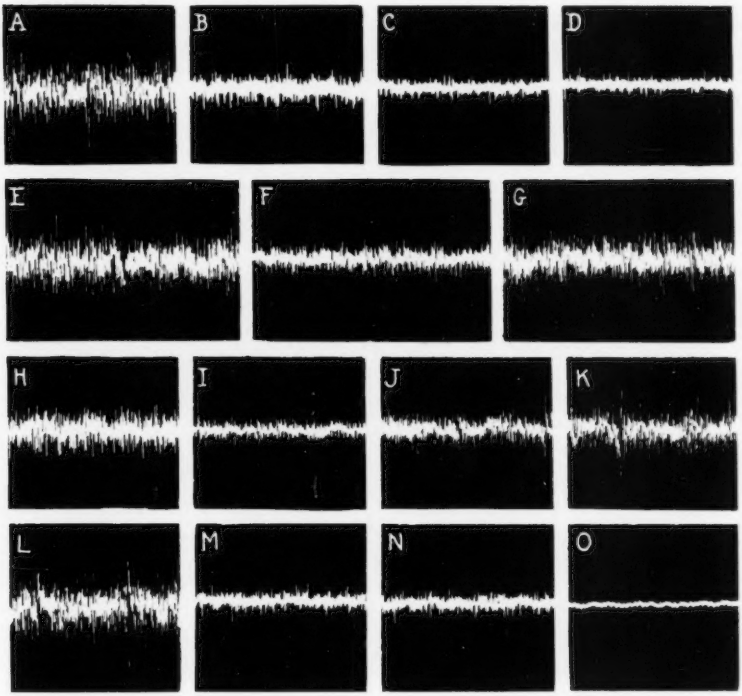

Fig. 10. Effect of various drugs on the spontaneous fibrillation of denervated muscle. Quadriceps, denervated 12 days. Adrenals ligated. A, background activity. B, 1.5 minutes after injection of 40 γ of adrenaline into aorta. C, 2 minutes after B. D, 1 minute after C. E, background activity. F, 3 minutes after stopping circulation to muscle by clamping aorta. G, 1 minute after restoring circulation. H, background activity. I, 30 seconds after injecting 20 mgm. of potassium chloride into aorta. J, 30 seconds after I. K, 30 seconds after J. L, background activity. M, 1 minute after injection of 20 mgm. of quinine hydrochloride into aorta. N, 7 minutes after M. O, control record from normal muscle of opposite side.

B. *Spontaneous fibrillary activity.* Within a week after denervation the muscles of a cat show spontaneous fibrillations, manifested electrically as continuous irregular asynchronous spike potentials (fig. 10). The effect of adrenaline on this activity was studied in 2 animals; in addition, the action of potassium, quinine hydrochloride and interruption of the circulation was

studied in 1 of these animals. In 1 animal 2 injections of 25 γ of adrenaline into the aorta resulted in a temporary (1 to 2 min.) increase in spike potentials followed by a marked decrease which lasted for 2 to 3 minutes, and then a gradual return to normal. In another animal 25 γ of adrenaline produced only the brief increase of spike potentials; 40 γ given shortly afterwards elicited only a marked decrease of the potentials (fig. 10). The decrease at the end of 3.5 minutes was more marked than that obtained by completely cutting off the circulation to the muscle for 3 minutes (fig. 10).

An intra-arterial injection of 15 mgm. of potassium chloride produced a striking but brief (1 to 2 min.) decrease of spike potentials (fig. 10).

An injection of 15 mgm. of quinine hydrochloride produced a decrease of the spikes which was evident in 15 seconds and still present after 8 minutes (fig. 10).

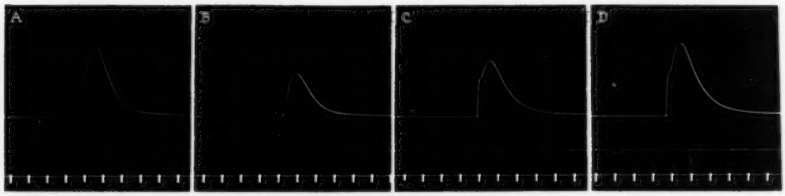

Fig. 11. Effect of potassium chloride on the response of denervated muscle to acetylcholine. Quadriceps, denervated 16 days. Adrenals ligated. Time: 5-second intervals. A, B, C and D were taken at 5-minute intervals. At second signal in each instance 10 γ of acetylcholine injected intra-arterially. At first signal in A, C, and D, 0.3 cc. of mammalian Ringer injected intra-arterially. At the first signal in B, 7.5 mgm. of potassium chloride in 0.3 cc. of distilled water injected intra-arterially.

C. *Contracture produced by acetylcholine.* 1. Action of quinine. Quinine decreases the response (contracture and contraction) of denervated muscle to acetylcholine, an observation previously made by Harvey (1939) and Oester and Maaske (1939).

2. Action of potassium. An intra-arterial injection of potassium chloride, given shortly before the injection of acetylcholine, decreases the response of the muscle to acetylcholine (fig. 11). This occurs both when the potassium chloride alone is not sufficient to cause an evident contraction and also when it causes a contraction. The response of the muscle to small doses of acetylcholine may be almost completely inhibited. The effect of potassium is gone in 5 to 15 minutes. In 1 animal it decreased both the contraction and the contracture responses to acetylcholine; although the contraction appeared more affected, the contracture took longer to return to normal (fig. 11).

Discussion. A few days after being denervated, mammalian skeletal

muscle shows, among other changes, 3 new phenomena: 1, a prolonged first contraction after a period of rest; 2, a spontaneous fibrillary activity; and 3, an increased sensitivity to acetylcholine and an increased tendency to respond to acetylcholine with contracture. These phenomena show a striking uniformity in response to several drugs. Adrenaline given shortly before acetylcholine will usually inhibit the response of the denervated muscle to acetylcholine, but under special conditions it may accentuate the response (Luco, 1939). Similarly, adrenaline usually inhibits the prolonged first contraction and the spontaneous fibrillation of denervated muscle, though at times it may have opposite effects. Potassium chloride injected intra-arterially inhibits all three phenomena. Quinine likewise inhibits all three phenomena. Frank, Nothmann and Guttmann (1933) found that calcium inhibits the response of denervated muscle to acetylcholine, and Langley (1915-16) reported that calcium inhibits the spontaneous fibrillations of denervated muscle. Calcium has been shown above to inhibit the prolonged first contraction. The response of denervated muscle to acetylcholine is increased by eserine as is the spontaneous fibrillary activity (Rosenblueth and Luco, 1937). The evidence that eserine produces an increase in the prolonged first contraction is not conclusive. Such uniformity of three phenomena which are produced by the same procedure strongly suggests that they represent different manifestations of the same change in the muscle.

The denervated muscle and the myotonic muscle show remarkable similarities (cf. table 1). The most remarkable is the occurrence of the prolonged contraction following a period of rest. This is seen in the myotonic muscle after contractions produced by nerve impulses or by mechanical or electrical stimuli. In the denervated muscle this prolonged first contraction occurs after electrical stimulation; whether it occurs after mechanical stimulation has not been determined. The prolonged contraction seen in denervated muscle is influenced by several drugs in almost exactly the same manner as the myotonic contraction. Adrenaline, calcium and quinine influence both responses in the same manner. Potassium at first glance appears to produce different effects, but in tests on denervated muscle the drug was injected intra-arterially, whereas in observations on myotonia it was given by mouth. Possibly the action of potassium chloride given by mouth over long periods of time would differ from its action when given intra-arterially. Prostigmin usually increases myotonia but no definite effect was shown in the denervated muscle. Brown and Harvey (1939), however, were unable to show that the muscles of goats with a condition which appears analogous to myotonia were more sensitive to eserine than normal muscles. The occurrence of the prolonged first contraction which behaves so much like the myotonic contraction and the numerous other points of similarity in the properties of

TABLE 1

	DENERVATED MUSCLE (TOWER, 1939)	MYOTONIA (RAVIN, 1939)
Mechanical excitability	Increased	Increased
Response to galvanic current	Increased excitability ACC equal to or greater than CCC Sustained contraction during passage of current	Increased excitability ACC often equal to or greater than CCC Sustained contraction during passage of current
Response to faradic current	Diminished excitability	(Cannot distinguish nerve from muscle excitation)
Spontaneous fibrillation	Present	Probably not usually present
Sensitivity to acetylcholine	Increased	Increased. Also reported as normal (Brown and Harvey, 1939)
Sensitivity to potassium chloride	Increased	Increased (Brown and Harvey, 1939)
Prolongation of first contraction after a period of rest	Seen following electrical stimulation	Seen following all types of stimulation
Effect on prolonged contraction of:		
1. Adrenaline	Decreases. May increase	Decreases. May increase
2. Quinine	Decreases	Decreases
3. Calcium	Decreases	Decreases
4. Potassium	Decreases on intra-arterial injection	Increases on oral administration
5. Eserine	No definite effect	Increases. No change also reported (Brown and Harvey, 1939)
Histology	Increase in sarcolemma nuclei and migration of nuclei to interior of fiber	Increase in sarcolemma nuclei. Occurrence of nuclei in interior of fiber also reported
	Atrophy	No atrophy in pure myotonia but atrophy is commonly associated with myotonia in dystrophia myotonica

the denervated and the myotonic muscle make it highly probable that one of the results of denervation is to produce a condition similar to that found in the myotonic muscle.

In a previous article (Ravin, 1940), myotonia was ascribed to a defect in the muscle rather than in the neuromuscular transmission. This conclusion was based on two considerations: 1, the similarity of myotonia and a group of representative contractures, and 2, an analysis of that characteristic of myotonia manifest in a duration of the myotonic contraction independent of the associated voluntary contraction. (Note the analogy between this characteristic and the independence of the prolonged first contraction of denervated muscle from subsequently induced contractions.) To this evidence for a muscular location of the defect in myotonia is now added the further evidence of 1, the similarity of the myotonic and denervated muscle, and 2, the observations of Brown and Harvey (1939) on goats, having a condition which appears to be the counterpart of myotonia of man, that the defect persists after curarization and denervation.

If myotonia is due to a defect in the muscle, is it a contracture or a contraction? It would seem that all that is necessary to settle this point is to determine the presence or absence of spike potentials. The problem is not simple: if no spikes are found, the instruments may be insensitive or the technique wrong. If spike potentials are found, they may be due to associated twitching, and the presence of a contracture is still not ruled out. Both the presence and the absence of action currents in myotonia have been reported recently. Brown and Harvey (1939) found them associated with the prolonged contractions occurring in their goats. On the other hand, Eichler and Hattingberg (1938) were unable to find them always in myotonic contractions. Brown and Harvey state that when the recording electrodes are applied so as to lead from a large proportion of the muscle fibers (as was done by Eichler and Hattingberg) the action currents may not be found during myotonic contractions, although a more localized lead, such as a concentric needle electrode, may reveal their presence. Lindsley and Curnen (1936) used needle electrodes in their studies on patients with myotonia and found action currents present. The conclusion appears safe that myotonic contractions are associated with action currents. It is not yet safe to exclude entirely the possibility of the occurrence also of a contracture.

The myotonic contraction behaves in many respects like the members of a group of representative contractures (Ravin, 1940). This observation presents three possibilities: 1, myotonia is a contracture; 2, myotonia is a contraction which, however, shows properties similar to a contracture; and 3, myotonia is a combination of a contraction and a contracture. The first possibility, that myotonia is a pure contracture, seems ruled out by

the work of Brown and Harvey, and Lindsley and Curnen. The third possibility requires, like the second, that the portion of myotonia which is a contraction should show many of the properties of the contracture. This is just what was found in the denervated muscle. The denervated muscle displayed a prolonged contraction, associated with action potentials, which behaved in many ways like the acetylcholine contracture. There is, then, an additional reason for believing that myotonic muscle and denervated muscle have some change in common. This change results in 1, prolonged contractions associated with action currents, and 2, increased tendency to respond by contracture. Since both responses behave in like manner to several drugs and conditions, the similarities which myotonia shows to contractures are easily understood, even if it is concluded that a contracture is not ordinarily present. It is furthermore not unlikely that some portion of the myotonic response may occasionally be a contracture. The question of whether myotonia is a tetanus or a contracture has lost much of its significance.

The ability of adrenaline to inhibit acetylcholine contractures has been attributed to a vasoconstriction which prevents the acetylcholine from reaching the muscle fibers (Dale and Gaddum, 1930). The action of adrenaline on the prolonged first contraction of denervated muscle furnishes evidence that it does act upon the muscle itself. Therefore, although the vasoconstrictor effect of adrenaline may play a part in checking the action of acetylcholine, there seems to be very little question that part of the action of adrenaline is on the muscle itself.

The difference in response of the quadriceps and gastrocnemius muscles to adrenaline may perhaps be the explanation of a discrepancy in observations on myotonia. In tests made mainly on the small muscles of the thumb, the author found that adrenaline always produced a marked decrease in myotonia (Ravin, 1940). This result differed from that reported by several previous observers. At the time no explanation could be offered; now it seems possible that in some muscles of myotonic patients the main effect of adrenaline is aggravation of the myotonia and that a fleeting improvement may be easily overlooked.

SUMMARY

If a denervated cat's muscle is stimulated electrically with single shocks, as from a condenser, the first contraction after a period of rest is more prolonged than succeeding contractions (figs. 1 and 2). The characteristics and the reactions to drugs of this prolonged contraction have been studied.

Adrenaline may decrease or increase the duration of the contraction (figs. 3–5). Potassium chloride (fig. 6), quinine hydrochloride (figs. 7 and 8), and calcium chloride (fig. 9) decrease its duration.

The spontaneous fibrillary activity characteristic of denervated muscle is decreased by intra-arterial injections of quinine hydrochloride and potassium chloride (fig. 10). Adrenaline may produce either an increase or a decrease of the fibrillations (fig. 10).

The response of denervated muscle to acetylcholine is decreased by quinine and potassium chloride (fig. 11).

That various drugs induce uniform effects on three of the characteristics of denervated muscle—spontaneous fibrillation, prolonged first contraction and changed response to acetylcholine—is emphasized.

The marked similarities in the characteristics of denervated muscle and myotonic muscle are pointed out, and the suggestion is made that one of the results of denervation is to produce a change in muscle similar to that which is present in myotonia.

I am indebted to Dr. W. B. Cannon for many helpful suggestions, and to Dr. A. Rosenblueth for taking the electrical records.

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EFFECTS OF QUININE ON MAMMALIAN SKELETAL MUSCLE

ABE RAVIN[1]

From the Department of Physiology in the Harvard Medical School

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The discovery by Wolf (1936) of the remarkable effect of quinine on myotonia has renewed interest in the actions of quinine on skeletal muscle. Harvey (1939) and Oester and Maaske (1939) investigated the problem with essentially similar results. The following important actions of quinine were observed: 1, an increase in the height of response to single maximal stimuli; 2, a depression of the response to tetanic stimulation; 3, an increase in the refractory period of the muscle; 4, a curare-like action; 5, an inhibition of the eserine-produced potentiation of muscle twitches, and 6, a depression of the response of denervated muscle to acetylcholine. Certain phases of the action of quinine seemed worthy of further investigation; many of the results here reported confirm and extend previously known actions of quinine.

METHODS. Cats were used in all experiments except those on the atrophy of denervated muscle. The anesthetic was dial (Ciba), 0.75 cc. per kgm., intraperitoneally. The quadriceps muscle was used for most experiments; a few performed on the gastrocnemius-soleus and tibialis anticus yielded similar results. The femur, or tibia, was fixed by means of drills, and the muscle tendons attached to the short end of a writing lever pulling against rubber bands. The femoral nerve was cut or crushed and stimulated distally by shielded silver electrodes. For direct stimulation of the muscle steel needles were inserted into the body and tendon. Rectangular waves from a multivibrator circuit were most frequently used for stimulation; the output was usually passed through a transformer before being applied to the nerves. Condenser discharges of various capacities and a thyratron circuit were also occasionally used for stimlation. All stimuli were maximal.

All injections of quinine were made into the abdominal aorta by retrograde cannulation of the inferior mesenteric artery. To increase the concentration of the drug going to the muscle the opposite iliac artery and the caudal branches of the aorta were ligated. The drugs employed were quinine hydrochloride, physostigmine salicylate, prostigmin (Roche), atropine sulfate, acetylcholine chloride (Merck), and curare (the Brazilian

[1] Medical Fellow of the National Research Council.

crude product). The quinine hydrochloride was dissolved in warm distilled water to make a 5 per cent solution.

White rats were used for the study of the atrophy of denervated muscles. In each animal the gastrocnemius-soleus muscle on one side was denervated by removal of a segment of the sciatic nerve. In both control and treated animals an equal number of right-sided and left-sided denervations were made. Half the animals received quinine injections; the other half were given injections of saline and served as controls. A 2.5 to 5.0 per cent solution of quinine hydrochloride was given subcutaneously. Aside from frequent local reactions most of the animals showed no ill effects. In one group of animals a fairly large dose of quinine hydrochloride was used and an occasional death shortly after the injection indicated that the dose was close to lethal. Since loss of weight complicates the results (Hines and Knowlton, 1934–35) an attempt was made to avoid any changes of weight, and animals showing a definite weight loss were discarded. At the end of the test period the animals were killed with carbon monoxide and the muscles dissected out and kept in weighing bottles until weighed.

Results. *Effect on indirectly stimulated muscle.* Rates of stimulation ranging from one every 30 seconds to 530 per second were investigated. The effect of quinine does not consist simple of an augmentation of the response to single stimuli and a depression of tetani. With small doses of quinine (5 to 15 mgm.) an increase in response for short periods (5–15 sec.) may be obtained with frequencies as high as 30 to 40 per second (figs. 1 and 3). At 60 per second the initial tension is usually unchanged or slightly increased by a small dose of quinine, but instead of being maintained the tension decreases as stimulation is continued. At rates of stimulation above 60 per second, even the initial tension may be lower than normal. The higher the rate of stimulation the more rapid the fall of tension as compared to normal. With larger doses of quinine, the response to high rates of stimulation may consist merely of a single twitch (fig. 4). If the muscle is stimulated at a rate sufficiently high (530 per sec.) to show the various stages of neuromuscular transmission (Rosenblueth and Cannon, 1940), it is evident that there is no selective augmentation or depression of any of the stages; from its initial height the tension falls more rapidly than normal and stages 3a and 3c are decreased in height or fail to appear (fig. 4). The action of an intra-arterial injection of quinine is at its maximum within 20 to 40 seconds and then gradually decreases so that most of the effect may be gone in 20 to 30 minutes.

Repeated doses of quinine further increase the response at slow rates of stimulation and at the same time lower the rate of stimulation required to show depression (figs. 1 and 3). With a sufficiently large dose of quinine (15 to 20 mgm. repeated 4 to 6 times at 8- to 10-min. intervals) the response to all rates of stimulation may be depressed even to the point of

extinction. The more frequent the rate of stimulation and the greater the dose of quinine the more evident the depression. When the response to nerve stimulation is depressed even at very slow rates of stimulation (1 every 15 sec.), it can be shown that the response of the muscle to direct stimulation is still much above the pre-quinine level (fig. 2). Moreover,

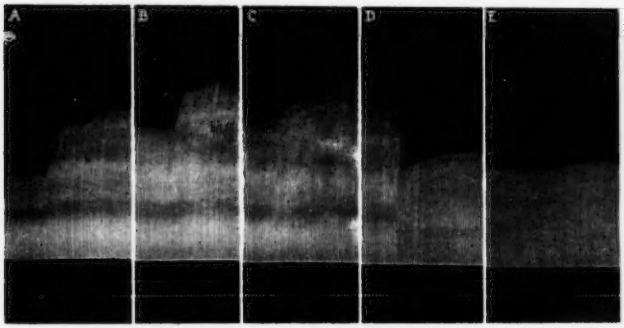

Fig. 1 Fig. 2

Fig. 1. Effect of quinine at slow rates of stimulation. Left quadriceps: femoral nerve stimulated at 2-second intervals by multivibrator circuit. Time: 5-sec. intervals.

A, 20 mgm. of quinine hydrochloride injected intra-arterially at signal.

B, 10 minutes later: 20 mgm. of quinine hydrochloride injected intra-arterially at signal.

C. 10 minutes after B; 20 mgm. quinine hydrochloride injected intra-arterially at signal.

D. 10 minutes after C; 20 mgm. quinine hydrochloride injected intra-arterially at signal.

E, 8 minutes after D; eserine (1 mgm. per kgm.) injected intra-arterially at signal.

Fig. 2. Effect of direct stimulation of muscle after quinine has markedly depressed response to indirect stimulation. Left quadriceps. Previous to A the animal had been given large doses of quinine; an initial increased contraction was followed by a decrease, and at A the responses are somewhat more than one-half the original height. Time: 30-sec. intervals.

A and B. Femoral nerve stimulated at approximately 5-sec. intervals. Quinine hydrocholoride (25 mgm. intra-arterially) at signal in A and (50 mgm. intra-arterially) at signal in B.

C. Three minutes after B. Direct stimulation of the muscle. Quinine hydrochloride (30 mgm. intra-arterially) at signal.

another injection of quinine will at times produce a further increase in the response (fig. 2). Very large doses are required to produce a depression of the response to slow, direct stimulation.

In some animals at slow rates of stimulation an injection of quinine may produce a brief decrease in response before augmentation.

At one stage in quinine action, rates of stimulation between 3 and 30 per

second may produce an increased initial tension, followed by a fairly rapid fall in tension and a slower rise (fig. 3C)—a phenomenon suggestive in some respects of the action of eserine. Also reminiscent of the action of eserine is the more complete tetanus obtained at slow rates of stimulation (30 per sec., fig. 3).

Harvey's observation that the increased response produced by quinine was not accompanied by any evidence of repetitiveness as judged by the action current was confirmed.

Each injection of quinine produced a marked fall of blood pressure followed by a gradual recovery. After an intra-arterial injection, however, the changes in the response of the muscle occurred before the blood-pressure changes and no correlation was evident between the two.

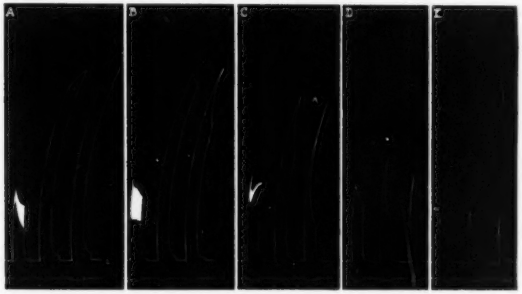

Fig. 3. Effect of quinine at intermediate rates of stimulation. Quadriceps; femoral nerve stimulated for 5 seconds every 2 minutes. Signal indicates duration of stimulation. Thyratron circuit. In each group the responses are to 30, 60 and 120 stimuli per second respectively.

A, before quinine. B, 1 minute after 15 mgm. of quinine hydrochloride. C, 4 minutes after B and 1 minute after 15 mgm. more of quinine hydrochloride. D, 4 minutes after C and 1 minute after 15 mgm. more of quinine hydrochloride. E, 4 minutes after D and 1 minute after another 15 mgm. of quinine hydrochloride.

Graham (1935) has shown that quinine produces changes in the excitability of nerves; therefore, the possibility that an action on the nerve might account for some of the changes seen on indirect stimulation must be considered. In one experiment the nerve potential showed no change in amplitude after injection of sufficient quinine to extinguish the muscular response. A more complete study is required, however, before the problem can be settled.

The quinine-eserine relationship in indirectly stimulated muscles. The response of a muscle after dosage with eserine will depend upon the frequency of stimulation. At slow rates (1 every 10 sec.) the response is usually increased; at frequencies of 3 to 6 per second the response is usually decreased; at higher frequencies the initial rise in tension is followed by a

prompt fall (Wedensky inhibition), then a slower rise, and finally a slow decline (plus-minus-plus response). Quinine given after eserine antagonizes these actions of eserine; it reduces the increase at slow rates of stimulation and the depression at higher rates of stimulation. If sufficient quinine is given the potentiation produced by quinine at slower rates of stimulation becomes evident (figs. 5 and 6).

If a small dose of quinine has been given, a sufficiently large dose of eserine (1 mgm. per kgm.) will produce an eserine potentiation at slow rates of stimulation. This can in turn be overcome by additional quinine.

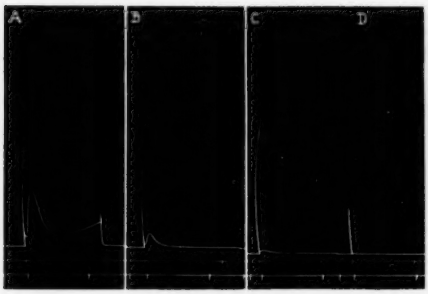

Fig. 4

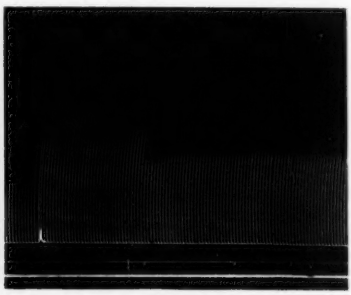

Fig. 5

Fig. 4. Effect of quinine at high rates of stimulation. Quadriceps; stimulation of femoral nerve at 530 per second by multivibrator circuit. Lower signal, time in 1-minute intervals; upper signal, beginning and end of stimulation. A, before quinine. B, 1 minute after 15 mgm. of quinine hydrochloride. C, 40 minutes after B; two injections of 15 mgm. each of quinine hydrochloride had been given meanwhile. D, 5 minutes after C and 1 minute after another injection of 15 mgm. of quinine.

Fig. 5. Antagonism of quinine and eserine. Quadriceps; femoral nerve stimulated at approximately 10-sec. intervals with condenser shocks. Atropine (1 mgm. per kgm.). Time: 5-sec. intervals. At first signal eserine (1 mgm. per kgm.) was injected into the jugular vein. At second and third signals 10 mgm. of quinine hydrochloride were injected intraarterially. At fourth signal eserine (1 mgm. per kgm.) was again given.

After a certain amount of quinine has been given, however, the usual doses of eserine produce no effect, or may produce slight depression. An exception is seen in those instances where quinine is given to the point of producing depression at slow rates of stimulation. In these circumstances an injection of eserine, if it is the first the animal has received, will produce a small potentiation (fig. 1). The depression which quinine produces at high rates of stimulation is, however, little if at all improved by eserine.

Very large doses of eserine will depress somewhat the quinine-produced potentiation. A more evident depression is seen following prostigmin;

injection of a fairly large dose may prevent or inhibit potentiation by a subsequent, not too large, dose of quinine (fig. 7). It is difficult to demonstrate any such effect with eserine.

Effect on post-tetanic potentiation. After a short tetanization the response of a muscle to single maximal stimuli is increased. Thus, indirect stimulation of the quadriceps at a rate of 530 per second for 10 seconds produces potentiation to subsequent single shocks. This potentiation is inhibited by quinine (fig. 8). If not too much quinine is given the post-tetanic potentiation may be restored or increased beyond normal values by eserine and then again inhibited by more quinine. Following large doses of quinine eserine is no longer effective. With large doses of quinine

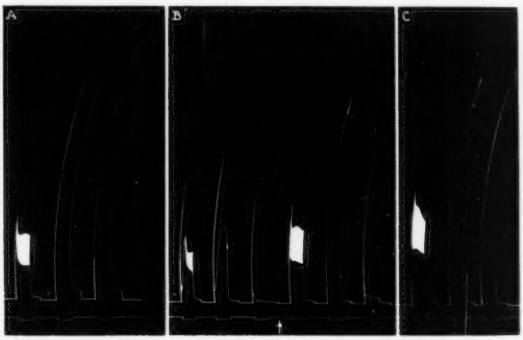

Fig. 6. Antagonism of quinine and eserine. Quadriceps; femoral nerve stimulated by thyratron for 5 seconds at 2-minute intervals. Signal indicates duration of stimulation. The order of stimulation was 30, 60 and 120 per second in each instance. A, before eserine. B, 10 minutes after giving eserine (0.3 mgm. per kgm. intravenously). At arrow quinine hydrochloride (15 mgm. intra-arterially). C, 8 minutes after B and just after another injection of quinine hydrochloride (15 mgm. intra-arterially).

also a secondary phase of depression of the twitches may occasionally be seen 1.5 to 2 minutes after the tetanus.

At the stage of quinine action where the response to nerve stimulation at slow rates is markedly depressed tetanic stimulation does not produce a potentiation of the response, such as occurs with curarized muscles. If, however, the muscle has rested for 20 to 30 minutes, the gradually improving response (due to decrease of quinine action) will show a post-tetanic increase.

Effect on curarized muscle. Previously reported observations (Harvey, 1939; Oester and Maaske, 1939), that quinine produces a potentiation in the response of a curarized muscle to slow rates of direct stimulation and that quinine further decreases the response of a partially curarized muscle to

nerve stimulation, are easily confirmed and can be predicted from what has been reported above. With small doses of quinine the increase in response at slow rates of stimulation is associated with a depression at more rapid rates. With increasing doses of quinine the depression occurs at lower frequencies and is finally seen at all frequencies. There are, however, two important differences from the indirectly stimulated muscle: 1, much

Fig. 7

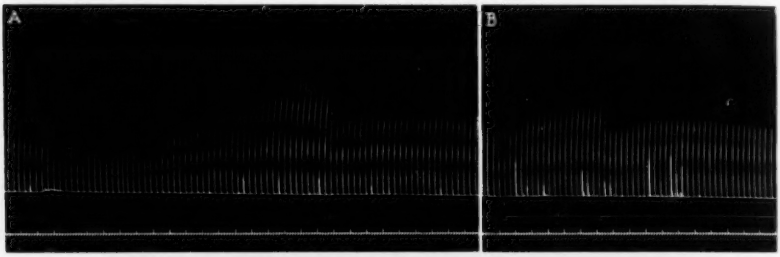

Fig. 8

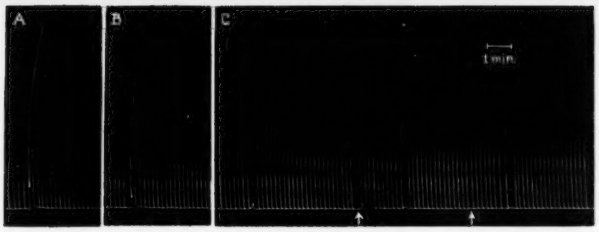

Fig. 7. Antagonism of quinine and prostigmin. Quadriceps; femoral nerve stimulated at 10-sec. intervals by condenser shocks. Atropine (1 mgm. per kgm.). Time: 5- and 30-sec. intervals. A, at first signal prostigmin (0.5 mgm.) was injected intra-arterially. At second signal quinine hydrochloride (20 mgm.) was injected intra-arterially. B, 22 minutes after A. At first signal quinine hydrochloride (20 mgm.) was injected intra-arterially and at second signal prostigmin (0.5 mgm.) was injected intra-arterially.

Fig. 8. Effect of quinine on post-tetanic potentiation of twitches. Quadriceps; femoral nerve stimulated every 10 seconds by multivibrator circuit. Atropine (1 mgm. per kgm.). Tetanic stimulation: 530 per second for 10 seconds. A, before quinine. B, 6.5 minutes after 10 mgm. of quinine hydrochloride. C, 20 minutes after B and 12 minutes after eserine (0.5 mgm. per kgm.). At arrows 10 mgm. of quinine hydrochloride were injected.

larger doses of quinine are required to produce the depression; and 2, the degree of depression obtained is not so marked (fig. 9).

Effects on denervated muscle. 1. Inhibition of spontaneous fibrillary activity. All denervated muscles show within several days after denervation a spontaneous fibrillation which is manifest electrically as a continuous irregular, asynchronous series of spike potentials. Quinine injected intra-arterially produces a marked inhibition of this fibrillary activity.

2. Effect on atrophy of denervated muscle. The marked atrophy which occurs in a denervated muscle has been attributed to its continuous fibrillary activity. If this is true, quinine might be expected to inhibit the atrophy. The results of a number of experiments carried out as indicated under "Methods" are given in table 1. It is evident that under the conditions of the experiment no obvious effect was noted.

3. Effect on the response to electrical stimulation. If a denervated muscle is stimulated with single shocks the first contraction following a

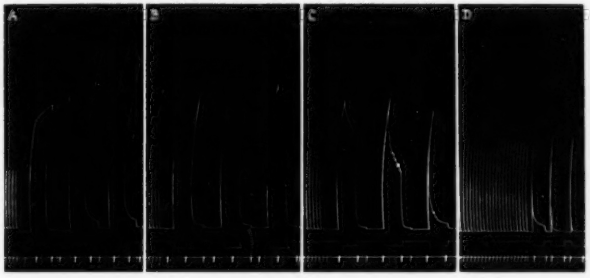

Fig. 9

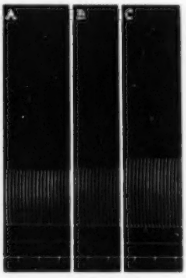

Fig. 10

Fig. 9. Effect of quinine on curarized muscle. Quadriceps; stimulated directly by multivibrator circuit after response to nerve stimulation had been abolished by curare. Lower signal: time in 5-sec. intervals; different spacing indicates different speed of drum. In each section the response to single stimuli at 5-sec. intervals is given just before the responses to tetanic stimulation. The upper signal indicates beginning and end of tetanic stimulations which were given at 2-minute intervals; the order of tetanic stimulations was 30, 72 and 125 per second in each instance. A, before quinine. B, 10 minutes after A; 10 mgm. of quinine hydrochloride had been given 9 minutes before and 15 mgm. 1 minute before. C, 8 minutes after B; 15 mgm. of quinine hydrochloride 7 minutes previously and 20 mgm. 0.5 minute previously. D, 20 minutes after C; 20 mgm. of quinine hydrochloride had been given 19 and 13 minutes previously and 30 mgm. had been given 7 minutes previously. At first signal 30 mgm. of quinine were injected.

Fig. 10. Potentiation of response to single maximal indirect stimuli by curare. Quadriceps; femoral nerve stimulated by multivibrator circuit as indicated. Time: 30-sec. intervals. A, before curare. B, 3.5 minutes after 0.005 cc. of curare injected intravenously. C, 7.5 minutes after B and 7 minutes after 0.01 cc. of curare.

period of rest is practically always prolonged, at times quite markedly. The second and succeeding contractions show only a slight prolongation which may decrease with continued stimulation. After a period of rest of 2 to 3 minutes a prolonged first contraction can again be obtained. Rosenblueth and Luco (1937) state that such prolonged contraction-remainders appear electrically as marked increases in the amplitude and frequency of the spike potentials in the spontaneous background. Injections of quinine inhibit the occurrence of the prolonged first contraction and may shorten the duration of succeeding contractions (fig. 11).

The denervated muscle shows the same sequence of responses to varying rates of stimulation as does the curarized muscle. With smaller doses of quinine the response to slow rates of stimulation is increased and the response to rapid rates of stimulation decreased. With successive doses of quinine the depression occurs at lower rates of stimulation and finally occurs at all rates. Again the doses of quinine required are much larger than in the indirectly stimulated muscles and the depression obtained is never so great. A plus-minus-plus response was not seen in either curarized or denervated muscles.

4. Effect on the response to acetylcholine. Quinine decreases both contraction and contracture resulting from intra-arterially injected acetylcholine.

TABLE 1

NUMBER OF ANIMALS		DURATION OF EXPERIMENT	DOSE OF QUININE HYDROCHLORIDE (GIVEN SUBCUTANEOUSLY TWICE DAILY)	PERCENTAGE OF ATROPHY OF DENERVATED MUSCLE AS COMPARED TO NORMAL	
Controls	Quinine treated			Controls	Quinine treated
		days	*mgm. per 100 grams*		
5	5	7	8	32.4	33.2
10	8	10	4	36.6	35.5
3	4	10	8	36.7	40.9
4	6	14	8	45.2	46.1

Discussion. The marked increase in response to single maximal indirect stimuli which eserine produces has been attributed to its anticholinesterase activity. It is therefore interesting to note that quinine also has a strong anticholinesterase activity (Matthes, 1930; Vahlquist, 1935). Although this activity is much less than that of eserine, the larger doses of quinine used may equalize the actual effects obtained in the animal. In addition to the increase in response to single maximal stimuli, certain other actions of quinine remind one of eserine: e.g., the plus-minus-plus response which is seen at moderate rates of indirect stimulation; the more complete tetanus obtained at slower rates of stimulation; the depression of response at high rates of stimulation. However, the absence of any evidence of repetitiveness in the action potentials and the occurrence of the quinine effect in curarized and denervated muscles show that the important influence of quinine is on the muscle and that the anticholinesterase activity probably plays little part in the observed phenomena.

The indirectly and directly stimulated muscles show the same sequence of events to varying rates of stimulation; that is, increase in response at slow rates and depression at rapid rates with depression occurring at progressively slower rates of stimulation as the dose of quinine is increased.

The increased response following both direct and indirect stimulation is undoubtedly due to an action of quinine directly on the muscle. This is indicated by the persistence of the increased response in the curarized and denervated muscles and by the absence of any evidence of repetitiveness in the action currents. The occurrence of the depression to indirect stimulation, however, is apparently not due to the changes in the muscle, for when the response to indirect stimulation is practically gone the muscle still responds very well to slow rates of direct stimulation and fairly well to moderate rates. The depression in the response of the indirectly stimulated muscle appear to be due to an effect of quinine on neuromuscular transmission. (The possibility of changes in nerve conduction have been mentioned, p. 231). Neuromuscular transmission and muscle response are apparently affected in a similar manner, but neuromuscular transmission is more sensitive to the action of quinine. The muscle and neuromuscular transmission behave as though, with quinine, each contraction is followed by a period of slow recovery during

Fig. 11. Effect of quinine on the prolonged first contraction of denervated muscle. Quadriceps, denervated 14 days. Adrenals ligated. Condenser shocks. Time: 5-sec. intervals. Series of 4 contractions were taken at 3-minute intervals. A and B before quinine. C, 4 minutes after 15 mgm. of quinine hydrochloride. D, 10 minutes after quinine. E, 22 minutes after quinine.

which another stimulus produces a decreased response; in the muscle, in addition, this phase is followed by a supranormal period during which the response is increased. The duration of the subnormal phase increases with increasing doses of quinine. It is interesting to note that Briscoe (1935) has shown that curare also, in small doses, will inhibit the response to rapid (150 to 160) rates of stimulation at a time when the response to slower (20 to 35) rates is only slightly affected.

Eserine and tetani are much less effective in overcoming the neuromuscular transmission block produced by quinine than that produced by curare. It appears probable that curare may have the quinine-like action of increasing the response of a muscle as a result of a direct action on the muscle. With very small doses of curare it is possible to obtain an increase in response of a muscle to slow (1 every 5 sec.) indirect stimulation. This was noted by Rossbach in 1876 and is shown in figure 10. In view of the marked effect of curare in inhibiting repetitive responses arising at the neuromuscular junction, it seems likely that this increased response is due to a direct action on the muscle.

In table 2 is shown the striking ability of quinine to antagonize the influence of eserine on skeletal muscle. In some respects it is more effective than curare, as in the actions on denervated muscle. The ability of eserine to antagonize quinine is, however, much less than its ability to antagonize curare, and in this respect the quinine-eserine antagonism is not as complete as the curare-eserine antagonism.

The negative results obtained in testing the effect of quinine on the atrophy of denervated muscle do not disprove the hypothesis of fibrillary activity as a cause of the denervation atrophy. The doses of quinine given were in some instances large, and they might be expected to have an effect on the fibrillary activity, but how marked the effect and how long it lasted

TABLE 2

ESERINE	QUININE
Increases the response to single maximal nerve stimuli	Inhibits or prevents the increase to single maximal nerve stimuli produced by eserine
Depresses the response to indirect tetanic stimulation and favors the occurrence of Wedensky inhibition at low frequencies of stimulation	Counteracts the depression to tetanic stimulation and the Wedensky inhibition at low frequencies produced by eserine
Has a decurarizing action	Has a curare-like action and inhibits the decurarizing action of eserine
Increases the post-tetanic potentiation of single maximal stimuli	Decreases the post-tetanic potentiation
Produces spontaneous twitchings of peripheral origin	Inhibits the spontaneous twitchings produced by eserine (Weiss, 1926)
Increases the spontaneous fibrillation of denervated muscle (Rosenblueth and Luco, 1937)	Decreases the spontaneous fibrillation of denervated muscle
Increases the response of denervated muscle to acetylcholine	Decreases the response of denervated muscle to acetylcholine

was not determined. It was felt, however, that the experimental method was sensitive enough to have shown some inhibition of the atrophy if the atrophy were a result of the fibrillation. If the failure to obtain inhibition of atrophy was due to inadequate dosage of quinine, the possibility of therapeutically inhibiting denervation atrophy in man by the use of quinine seems out of question. In a recent preliminary report Solandt and Magladery (1940) state that they were unable to influence the atrophy of denervated muscles of albino rats with quinidine.

Of the many ways in which the denervated muscle resembles the myotonic muscle the prolongation of the first contraction after a period of rest is striking and significant. The characteristics of this prolonged contraction, and the ability of quinine and several other substances (po-

tassium chloride, adrenaline and calcium chloride) to inhibit it, form the basis of another report in which the marked similarities between the denervated and myotonic muscles are discussed in full.

SUMMARY

Quinine in small doses increases the response of a muscle to slow rates of direct or indirect stimulation while depressing the response to high rates of stimulation. With increasing doses of quinine the rate of stimulation required to show depression decreases, and finally depression is evident at all rates of stimulation (figs. 1, 3, 4, 9). Larger doses of quinine are required to produce the depression in directly stimulated muscle than in indirectly stimulated muscles; the probable explanation for this is discussed (p. 237).

Quinine antagonizes the actions of eserine on skeletal muscles as effectively as, and in some instances more effectively than, curare (table 2). Eserine is, however, not as effective in antagonizing the actions of quinine as it is in antagonizing the actions of curare.

Quinine inhibits the fibrillation of denervated muscle but an attempt to inhibit the atrophy of denervation in the gastrocnemius-soleus muscle of the white rat was unsuccessful (table 1).

The prolonged first response to electrical stimulation which a denervated muscle shows and which has many properties in common with myotonic contractions is inhibited by quinine (fig. 11).

I am indebted to Dr. W. B. Cannon and Dr. A. Rosenblueth for their interest and many helpful suggestions.

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THE RELAXATION EFFECT OF ACETYLCHOLINE ON THE OVIDUCT OF THE RABBIT IN RELATION TO HORMONAL STATUS

A. M. DAVIDS[1] AND M. B. BENDER[2]

From the Laboratories of the Mount Sinai Hospital, New York, N. Y.

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Although numerous studies have been made on the effects of adrenaline on the tube and uterus, there are few investigations on the action of acetylcholine on the Fallopian tube. Li, (1935) in his experiments on the isolated oviduct of the monkey found inconstant and variable extent of contractions with acetylcholine perfusions. He found no definite relationship between the adrenaline or acetylcholine response of the Fallopian tube and the menstrual cycle.

In a previous communication (Davids and Bender, 1940) it was reported that intravenous injections of epinephrine produce contractions of the intact oviduct of the rabbit. These contractions are more pronounced during estrus or after the administration of estrogenic substances and are diminished on treatment with testosterone propionate.

At present we call attention to the effects of acetylcholine on the intact oviducts of rabbits in different hormonal states. In many instances comparative studies were made between the two "reciprocally active" substances, namely, adrenaline and acetylcholine.

METHOD. Mature female rabbits, weighing on the average of 3.2 kgm. were used in all experiments. The tubal contractions were recorded by the kymographic technique as described by Rubin (1927), Wimpfheimer and Feresten (1939), and Davids and Bender (1940).

In several rabbits simultaneous records of uterine contractions were made. The technique was that devised by Reynolds, (1930). A tubular balloon was inserted into the uterine cavity and connected to a bellows. Water was used to transmit the fluctuations in the balloon caused by uterine contractions.

The acetylcholine solutions were prepared from the powder (Merck & Co.) dissolved in normal saline. A constant volume of 1 cc. and constant speed of two seconds' injection were used in all trials. The eserine salicylate

[1] Dr. Hiram N. Vineberg Research Fellow in Gynecology.

[2] Aided by grants from Josiah Macy, Jr. Foundation and the Dazian Foundation for Medical Research.

used to potentiate, and the atropine sulfate used to block the actions of acetylcholine, were given intravenously. In several rabbits bilateral adrenalectomy was performed through the abdominal surgical wound. In many instances the effects of acetylcholine on the tubal contraction during anestrus were compared with those observed during estrus or following the injections of estrogenic or androgenic hormones. Many side effects of acetylcholine such as lacrimation, salivation and defecation were noted and used as an indication of the degree of acetylcholine intoxication.

RESULTS. I. *Anestrus.* The oviducts of twenty anestrus rabbits were insufflated and tested with intravenous injection of acetylcholine.

In eight of these rabbits intravenous injection of 10 gamma of acetylcholine produced a frank relaxation of the tube (fig. 1). With previous eserinization, 0.6 mgm. injected intravenously, the relaxation was somewhat more pronounced.[3] Eserine alone produced no visible alteration in the tubal contractions. Atropine sulfate 1 mgm., injected intravenously one to ten minutes before the acetylcholine, blocked this relaxation. In

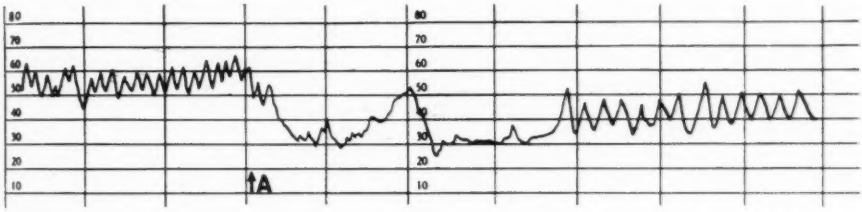

Fig. 1. Tubal contractions of a rabbit in estrus. Weight 3.5 kgm. At *A*, acetylcholine 20 gamma was injected intravenously. Abscissa, time in minutes.

two instances the inhibitory effect of acetylcholine could be demonstrated in the oviduct severed from the uterus, the insufflation of the CO_2 being made through the fimbriated end. The acetylcholine effect usually appeared from twelve to twenty seconds after the administration of the drug.

In four rabbits the relaxation effect of acetylcholine could be demonstrated only after a persistent contraction of the oviduct obtained by intramuscular injection of adrenaline (fig. 2). Adrenaline 0.1 to 0.3 mgm. per kgm. of body weight when injected intramuscularly produced a sustained contraction or spasm in the oviduct which lasted at times as long as fifteen minutes. This reaction to intramuscular adrenaline simulated that observed after intravenous injections. The response to intramuscular injection of adrenaline was increased during estrus or following administration of estrogenic substances and decreased or prevented after the injection of testosterone propionate.

[3] All tests with acetylcholine were made in uneserinized rabbits unless otherwise stated.

The relaxation effect of acetylcholine could also be demonstrated by intramuscular injections. Potentiation with eserine was difficult to elicit. Atropine sulfate always annulled the acetylcholine responses. The duration of the blocking action, however, depended on the amount of atropine given. One milligram of atropine sulfate given intravenously prevented the acetylcholine effect for ten to fifteen minutes.

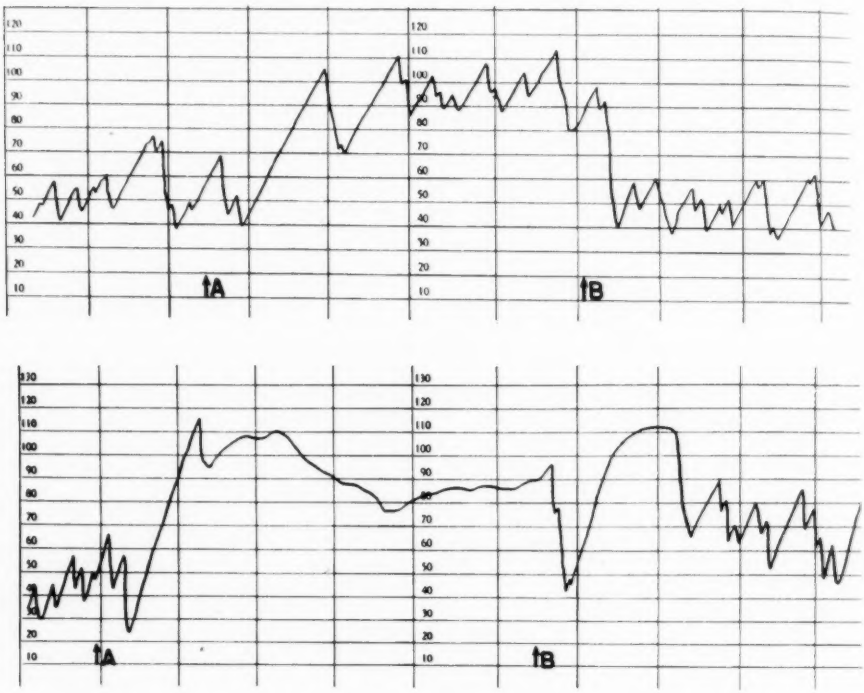

Fig. 2. (a) Upper: tubal contractions of rabbits in anestrus. Weight 3.3 kgm. At *A*, adrenaline 0.7 mgm. was injected intramuscularly. At *B*, acetylcholine 20 gamma was injected intravenously.

(b) Lower, weight 3.2 kgm. At *A*, adrenaline 0.8 mgm. was injected intramuscularly. Note the tubal spasm characterized by lack of fluctuations in the curve. At *B*, acetylcholine 10 gamma was injected intravenously.

In four rabbits acetylcholine in 10 or 20 gamma doses produced a contraction of the tube of the same magnitude as that obtained with adrenaline. This contraction sometimes appeared thirty seconds after the injection of the drug. The contraction was abolished by atropinization but not by bilateral adrenalectomy in one experiment. Two of these

rabbits exhibited both contraction and relaxation of the tube with acetylcholine, "diphasic effects"; the other two rabbits showed no relaxation effects.

In four other rabbits acetylcholine produced neither relaxation nor contraction of the tube. Eserinization or increasing the dosage of acetylcholine was also without effect.

2. *Estrus.* Eight rabbits were tested during estrus. In six of these estrus rabbits acetylcholine 10 gamma per rabbit, when injected intravenously produced a precipitous drop in the level of tubal contraction curve. Eserine salicylate slightly enhanced this relaxation while atropine (1 mgm.) always blocked it. A synchronous record of uterine contraction by

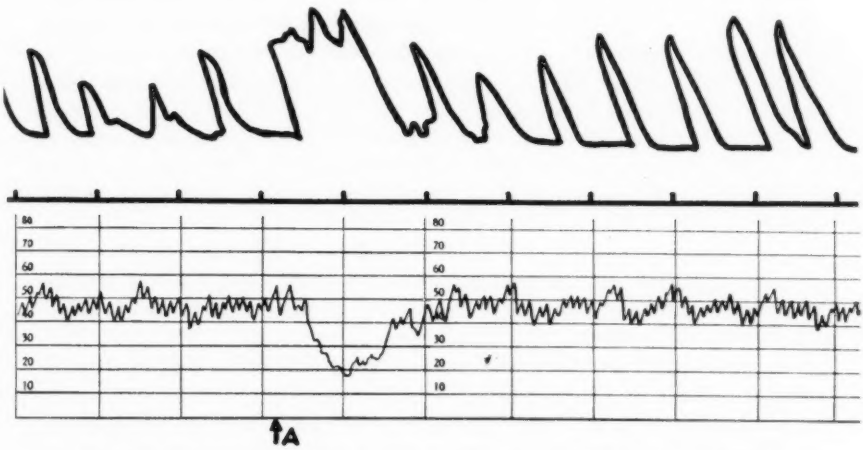

Fig. 3. Simultaneous record of uterine (upper tracing) and tubal (lower tracing) contractions. Rabbit in estrus. Weight 3.3 kgm. At *A*, acetylcholine 10 gamma was injected intravenously. Note contraction spasm in uterine and relaxation in tubal curves. Abscissa, time in minutes.

the Reynolds balloon method was made in four rabbits. Characteristic spontaneous uterine contractions were noted. The frequency of the uterine contractions was less than the tubal contractions which were simultaneously recorded. Intravenous injections of acetylcholine 10 gamma per rabbit, in these preparations, resulted in a contraction of the uterus and simultaneous relaxation of the tube (fig. 3). These results indicate that in general acetylcholine has a motor effect on the uterus and inhibitory action on the oviduct.

In two of the estrus rabbits acetylcholine produced neither motor nor inhibitory effect on the tubal contraction curve.

3. *Estradiol benzoate.* Four anestrus rabbits were injected intramus-

cularly with divided doses of estradiol benzoate (Roche Organon), the total doses varying from two to four thousand international units. Two to three days later these rabbits were tested with acetylcholine.

In three of these, intravenous injection of acetylcholine (10 gamma) produced a relaxation of the tube and in one a contraction. The inhibitory effect of acetylcholine was particularly conspicuous when the oviduct was in spasm due to intramuscularly injected adrenaline.

4. *Testosterone propionate.* Eleven anestrus and estrus rabbits were injected intramuscularly with testosterone propionate (total doses 100 to 200 mgm.) over a two week interval.

In only one of these rabbits did intravenous injection of acetylcholine produce a relaxation of the tube and this was not conspicuous. In four rabbits the intravenously injected acetylcholine (10 gamma per rabbit) produced a transient contraction of the tube, and in six rabbits acetylcholine was without effect. The contraction produced by acetylcholine was always blocked by atropine.

Adrenaline injected intramuscularly in the same doses which produced a prolonged and almost spastic contraction in the anestrus, estrus or estradiol benzoate treated rabbits, was conspicuously without effect in the rabbits treated with testosterone propionate. Only in two rabbits so treated did intramuscularly injected adrenaline (0.8 to 1.2 mgm.) produce a slight and sustained contraction of the tube. It seemed that testosterone propionate interfered with the actions of the injected adrenaline and acetylcholine on the oviduct.

5. *Castration.* Three rabbits were surgically castrated and tested with acetylcholine four weeks hence.

In all three rabbits castration did not alter the relaxation effect of intravenously injected acetylcholine. The degree of relaxation was not as great as in the anestrus and estrus rabbits before castration. Eserine and atropine exhibited the same properties as recorded previously.

6. *Injection with pregnant urine.* Three anestrus rabbits were injected intravenously with urine of pregnant women. Intravenous injections of acetylcholine in these rabbits three and four days later produced a relaxation effect in two of the rabbits.

Discussion. The foregoing experimental data reveal several interesting facts. The first is that acetylcholine injected intravenously in small doses into the uneserinized rabbit has an inhibitory influence on the muscle tone of the oviduct and excitatory action on the uterus. The relaxation property of acetylcholine is directly antagonistic to that of adrenaline on the same organ. These reciprocal chemical effects confirm the assumption that the sympathetic nerves exert a stimulating or contractile while the parasympathetic nerves exert an inhibitory influence on the Fallopian

tube. Incidentally the relaxation effect of acetylcholine on the tube is another example of inhibitory action of this drug, the others being dilatation of blood vessels, relaxation of ileocolic sphincter and inhibition of heart action.

Second is the influence of sex hormones on the inhibitory property of acetylcholine. It should be noted that during estrus and following the intramuscular injection of estrogens most of the effects produced by acetylcholine are inhibitory, whereas following treatment with testosterone propionate the relaxation influence of acetylcholine on the tube is negligible. From these and previous observations (Davids and Bender, 1940; Rubin and Davids, 1940) it would appear that testosterone propionate suppresses while estrogens potentiate the general activity and reactivity of the oviduct of the rabbit to adrenaline and acetylcholine.

The third is the less common contractile effect of acetylcholine on the oviduct. This motor effect may be explained by the well known observations that acetylcholine stimulates the adrenals and autonomic ganglia causing a secretion of adrenaline from adrenal gland and "sympathin" from sympathetic nerve endings throughout the body; the liberated adrenergic hormones, more stable than the acetylcholine injected into the blood stream, in turn act on the oviduct and thus produce a contraction. In some instances the relaxation effect of the injected acetylcholine and contractile effect of the reflexly liberated adrenergic substances neutralize each other, and no reaction is obtained. Still in other cases both inhibitory and motor effects are obtainable, "diphasic effects". Similar "diphasic effects" of acetylcholine have been found in experiments on the denervated iris of the cat by Bender and Weinstein (1940). As in case of the denervated iris all of the acetylcholine effects on the oviduct were abolished by atropinization. The fact that adrenalectomy did not annul the motor effect of acetylcholine on the tube suggests that a substance reflexly liberated at sympathetic nerve endings contracts the oviduct.

An alternate explanation for the double reactions of acetylcholine is that the smooth muscle cells of the oviduct may contain hypothetical chemical "receptive substances" (Langley, 1905) which unite with acetylcholine. When the injected acetylcholine combines with the excitatory factor, there results a contraction. When it unites with the inhibitory factor, there results relaxation.

Any chemical change in the hypothetical receptive substance such as brought about by treatment with sex hormones may alter the response to acetylcholine as observed in our experiments. Thus a contraction instead of relaxation of the tube may occur after treatment with testosterone propionate. The same theory may explain the reversal of uterine response to adrenaline during pregnancy (Kennard, 1937).

SUMMARY

1. The effects of acetylcholine on the intact oviduct of rabbit were studied by the tubal insufflation method.

2. Acetylcholine has in general an inhibitory effect on the oviduct which is directly antagonistic to the motor action of adrenaline on the same organ.

3. While acetylcholine relaxes the tube it simultaneously produces a contraction of the uterus in the estrus rabbit.

4. The hormonal state affects the tubal response to intravenously injected acetylcholine.

5. In some rabbits acetylcholine produces a contraction in the tube; in others both relaxation and contraction, "diphasic effects", are found.

We are indebted to Dr. I. C. Rubin for his valuable suggestions and aid in these experiments.

We wish to thank Roche Organon Company for the Testosterone Propionate (Neo-Hombreol) and Estradiol Benzoate (Dimenformon Benzoate) used in these experiments.

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EXPERIMENTS ON THE VASCULAR SUPPLY OF THE RABBIT'S HYPOPHYSIS[1]

CHANDLER McC. BROOKS AND I. GERSH

From the Departments of Physiology and Anatomy, the Johns Hopkins University

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Recent increases in knowledge of the functional relations between hypothalamus and hypophysis have taken place largely as a result of surgical interruption of the connections between these parts. This is especially true of the studies on the rôle of the neurohypophysis in the control of water balance (1). The technique has also been used in determining the importance of hypothalamico-hypophysial connections in the discharge of certain hormones from the anterior lobe (2, 3). The functional disturbances caused by these operations have been attributed by almost all workers to severance of neural connections between the hypothalamus and infundibular process or the anterior lobe. Yet the possibility exists that as a result of section of the hypophysial stalk or of the placing of electrolytic lesions in its neighborhood vascular injury may take place. Thus the functioning of cells in the hypophysis might be affected seriously. This was, in fact, the basis for the belief of Mahoney and Sheehan (4) that nervous connections between hypothalamus and pars nervosa in the dog may not be important in the control of water intake and output. Others have considered the possible significance of vascular disturbances in the region of the stalk produced by operative injury but regarded them of no great importance (5). The experiments described in this paper were carried out in order to determine the effect on the hypophysial vascular bed of depriving the gland of one or more sources of arterial blood.

In the rabbit Wislocki and King (6) have shown that arterial blood reaches the hypophysis through a large number of arteries of small caliber derived from the circle of Willis. These afferent vessels enter the gland either directly or after passing along the stalk. Some of them bear blood which has already passed through a capillary bed in the stalk or median eminence. Several of the arteries are large enough to be seen with the unaided eye and can be ligated or cut with ease. There are two or three such vessels which arise on each side from the circle of Willis near the point where it is joined by the internal carotid artery. These pass medially

[1] This work was aided in part by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.

toward the stalk. More caudally there are two other vessels, one on each side, which leave the arterial circle near the basilar artery and pass forward toward the hypophysis. In addition to these macroscopic vessels derived from the circle of Willis a pair of arteries arise from the internal carotids and enter the posterior lobe of the hypophysis. In summary, the chief sources of arterial blood to the hypophysis are the following: 1, vessels which pass in or around the stalk toward the pituitary gland; 2, lateral twigs which pass from the circle of Willis toward the hypophysis independently of the stalk; 3, posterior twigs arising from the caudal part of the circle of Willis to enter the hypophysis directly; 4, arteries of the posterior lobe derived directly from the internal carotid arteries.

PROCEDURE. The following procedures were used to test the importance of each of the above sources of arterial blood in the maintenance of the capillary bed of the hypophysis.

Adult rabbits were killed with illuminating gas. Immediately thereafter the hypophysis was deprived of one or more sources of arterial blood by cutting the vessels concerned or by occluding them with silk ligatures or silver clips. This was followed by injection of India ink (diluted with an equal volume of water) through the aorta at a pressure of approximately 120 mm. of Hg. When the injection was judged to be complete the hypophysis was dissected out carefully, fixed in formalin, embedded in celloidin and sections 100 μ thick were cut serially. The sections were studied unstained and the injected areas mapped accurately. Maps of several glands deprived of the same arterial supply were superimposed and the composite was compared with the blood vessel pattern of three normal glands injected in the same way but with no interference to their blood supply. Particular attention was paid to capillary anastomoses between the different lobes of the hypophysis.

The vascular operations preceding the injection of India ink were carried out as follows:

1. The stalk alone was cut in seven rabbits. Arterial blood could reach the hypophysis by way of the lateral and posterior twigs from the circle of Willis and from the arteries of the posterior lobe which have their origin in the internal carotids.

2. The lateral twigs from the circle of Willis and the adjacent internal carotid arteries were interrupted in six rabbits. Blood could then reach the hypophysis through the stalk vessels, through the posterior twigs from the circle of Willis (by way of the basilar artery) and through the posterior lobe arteries.

3. In three rabbits the stalk and lateral twigs were cut. After this operation blood could still reach the hypophysis through the posterior twigs and the posterior lobe arteries.

4. All vascular connections between the circle of Willis and the hypoph-

ysis were cut in three rabbits. In order to make sure that the stalk and blood vessels had been completely severed the brain was raised from the diaphragma sellae turcicae. Under these circumstances blood could reach the hypophysis only by way of the posterior lobe arteries from the internal carotids.

5. The basilar and both common carotid arteries were ligated and sectioned in two rabbits. Theoretically no blood should reach the hypophysis following this procedure.

RESULTS. Excellent capillary injections that were almost complete were obtained in two of the three normal glands. The anterior lobe of the third was poorly injected in the region of its anterior inferior surface. In all cases the capillary bed of all parts of the hypophysis was quite continuous. Capillaries pass between infundibular process and the pars intermedia, between the latter and the anterior lobe around the edges of the residual lumen of Rathke's pouch, and between the infundibular stem and the pars distalis. Of the experimentally treated animals, the only other instance of a similarly injected hypophysis was one whose lateral twigs had been cut. In all other cases (except the two of group 5 in which the entire arterial circulation to the hypophysis was blocked) the capillaries were injected incompletely. In order to evaluate the irregularities in the extent to which vessels might be expected to be injected, diagrams of the injected region of the gland in different animals of each group were superimposed. The resulting composite picture was believed to represent more accurately the true extent of the entire capillary bed in the hypophysis following each type of opertaion. The composite capillary bed derived in this way is described below.

1. *Stalk alone cut.* The capillaries were injected as completely as in normal glands. A small number of capillary anastomoses between pars intermedia and the anterior lobe, and between the latter and the infundibular stem could be seen clearly in one specimen in which the only capillaries of the anterior lobe injected lay adjacent to the pars intermedia and the stalk and were continuous with their vessels. In this preparation they extended for a short distance only into the anterior lobe.

2. *Lateral twigs alone cut.* The capillaries were as completely injected as in normal glands.

3. *Stalk and lateral twigs cut.* The capillaries were fully injected as in the normal glands.

4. *Stalk, lateral twigs, posterior twigs and other vascular connections with circle of Willis cut.* The posterior lobe vessels were fully injected and they had the same appearance as in normal glands. Capillaries from pars intermedia and infundibular stem passed for a short distance into the pars anterior. They spread out from around the edge of the residual lumen of Rathke's pouch so as to cover its whole anterior surface. In two glands

one or more large veins from the anterior lobe were injected because of the back pressure of the injection fluid in the venous system; the capillaries in the surrounding region contained ink particles.

5. *Basilar and common carotid arteries cut.* No capillaries in the hypophysis were injected.

Discussion and conclusions. One of the most prominent aspects of the observations described above is that a free anastomosis exists between the capillary beds of different, perhaps widely separate vascular regions of the pars anterior. For example, the integrity of only two of the large number of arteries normally present (such as posterior twigs from the circle of Willis) may be sufficient to assure the access of India ink to the capillaries of the whole lobe.

Another conclusion substantiated by these experiments is that injections of the various parts of the hypophysis can be obtained after destruction of the arterial and portal circulation in or around the stalk. In experiments in which the stalk was cut (even when the lateral twigs also were severed) the anterior lobe and the pars intermedia appeared quite well injected. The infundibular process and the pars intermedia were thoroughly injected even when the whole arterial supply of the hypophysis was interrupted except for those branches passing to the posterior lobe from the internal carotid arteries. To the limited extent to which this type of evidence is applicable, the stalk circulation does not appear to be essential to the completeness of hypophysial circulation.

One is impressed by the limited number of capillary anastomoses between pars intermedia and pars anterior. This has been demonstrated experimentally by Morato (7). The pars intermedia, so far as its circulation pattern is concerned, appears to be more closely related to the pars nervosa. This may be related to the fact that the surface between these two parts of the hypophysis is, of course, greater than that between pars intermedia and anterior lobe and offers a richer opportunity for capillary anastomoses.

Further implications are limited because the method of vascular injection is an indirect one. It is quite possible, for example, that the existence of a potential capillary circulation may be demonstrated by India ink injection for a region whose major blood supply is occluded and in which the rate of blood flow may be reduced to such a degree as to be virtually equivalent to complete stasis. On the other hand, the potential circulation may become a reality through reorganization of the vascular bed. The number of variables is too great to be controlled and clarified merely by the technique of India ink injections of acute preparations. Chronic experiments should throw more light on the situation. It is pertinent to recall that in some stalk-cut rabbits which survived a long time after operation Brooks (2) found that a small central scar developed in the anterior lobe. In a number of cases one or more of the arterial twigs

which run with or along the stalk were cut inadvertently as the stalk was being sectioned. Such animals sometimes showed signs of anterior lobe deficiency. In these cases, large areas of degeneration in the pars distalis were found at autopsy.

In spite of the limitations of the method employed it seems proper to conclude that stalk section alone should not interfere with the blood supply of the infundibular process in a surviving rabbit. Stalk section may possibly modify the circulation in the anterior lobe. If there are any ill effects of such an operation they may be minimized in a surviving rabbit by the free capillary anastomoses which exist within the gland. These conclusions have a clear bearing on the interpretation of the disturbances of water exchange and of certain reproductive mechanisms that follow stalk section. Since the effects of this operation on the circulation within the hypophysis are relatively unimportant, the abnormalities of function caused by stalk section must be related instead to interruption of the nerve pathways.

SUMMARY

In a series of acute experiments the various components of the vascular supply to the hypophysis of the rabbit were occluded singly or in various combinations. To determine the effects of these procedures the capillary bed of the gland was injected with India ink.

It was found that a free anastomosis exists between the capillary beds of different regions of the pars anterior. When only a portion of the arterial supply is left intact the capillaries of the whole lobe are perfused. Transection of the hypophysial stalk does not impair the completeness of the injection. Capillary anastomoses between this lobe and the pars intermedia are few in number. The pars nervosa and pars intermedia can be perfused completely by way of the posterior lobe arteries which run to the caudal aspect of the gland from the internal carotids.

Since the effect of stalk transection on the circulation within the hypophysis is relatively unimportant, the abnormalities of function caused by this operation must be due to interruption of nerve pathways.

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THE AVAILABILITY OF dl-THREONINE AND dl-ALLOTHREONINE FOR THE FORMATION OF CARBOHYDRATE[1]

W. KNOWLTON HALL, J. ROY DOTY AND A. G. EATON

From the Departments of Biochemistry and Physiology, Louisiana State University School of Medicine, New Orleans

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It was first demonstrated by McCoy, Meyer, and Rose (1) that an α-amino-β-hydroxy-n-butyric acid was a naturally occurring, essential amino acid. It was later demonstrated that the d(−) threonine, as it was named, was the naturally occurring form (2) and the only form essential for growth of the rat (3). Little is known as to the metabolic pathways followed by this amino acid or its isomers, though Knoop (4) has developed a theory concerning the metabolism of aliphatic β-hydroxy-amino acids. Snyder and Corley (5) on injecting dl-allothreonine concluded that it was not significantly deaminized.

The purpose of the present study was to determine whether or not dl-threonine or dl-allothreonine is available for the formation of carbohydrate in the rat.

EXPERIMENTAL. Two procedures were used to determine carbohydrate formation. The first, following the method used by Butts, Dunn and Hallman (7), was to determine the amount of liver glycogen produced after feeding the amino acids to rats previously maintained for 2 days on a low residue diet. Female rats 140 to 180 grams in weight were fasted for 24 hours and then fed a definite amount of the synthetic amino acid every two hours over a four or eight-hour period. The amount administered was always in excess of the absorbing capacity of the gastro-intestinal tract of the animal. The animals were allowed access to distilled water at all times and to filter paper throughout the 24 hour fasting period. At the conclusion of the experimental period sodium amytal was injected, the livers were removed, and glycogen determinations were made by the method of Good, Kramer and Somogyi (6). These results are given in table 1.

Since we found in a preliminary experiment that more glycogen was

[1] Aided by a grant from the Committee on Scientific Research, American Medical Association.

A preliminary report of these studies was presented before the fifty-second meeting of the American Physiological Society at New Orleans, March 13, 1940.

formed in eight hours than in four, the longer period of time was used in subsequent experiments. In these preliminary studies the rats were given 0.5 gram of amino acid per feeding. The almost universal diarrhea among the experimental animals and the distended, hyperemic conditions of the gastro-intestinal tract at the end of the experimental period indicated that the dose of amino acid was too large. Reduction of the dose to 0.3 gram per feeding avoided the diarrhea and resulted in an increased formation of glycogen. One group of four rats which was fed the larger dose of dl-threonine, but which did not develop diarrhea is included in the table (group C).

The data clearly demonstrate that both dl-threonine and dl-allothreonine form glycogen in the rat. The differences between the amount of glycogen formed from dl-threonine or dl-allothreonine are probably not significant. If one animal in group D (dl-allothreonine) which produced only 0.11 per cent of liver glycogen is excluded, since the result in that instance is ob-

TABLE 1

Per cent of glycogen in rat livers

GROUP	NUMBER OF RATS IN GROUP	COMPOUND FED	RANGE	AVERAGE
A	12	Distilled water (control)	0.02-0.31	0.12
B	6	dl-Threonine, 0.3 gram every two hours for 8 hour period	0.34-0.83	0.62
C	4	dl-Threonine, 0.5 gram every two hours for 8 hour period	0.69-1.21	0.90
D	8	dl-Allothreonine, 0.3 gram every two hours for 8 hour period	0.11-0.92	0.58

viously out of line with the other figures from the group, the average in this case becomes 0.64 per cent. This may be compared with 0.62 per cent of glycogen found for dl-threonine (group B). We doubt that any very significant comparison can be made between our figures and those of other investigators since there seems to be some difference between values for liver glycogen obtained with rats of different strains under similar experimental conditions and with similar previous nutrition. Furthermore, it would seem to us that direct comparisons of the glycogenic action of the various amino acids by this method would presuppose the same or very nearly the same rates of deamination. Snyder and Corley (5) found little, if any, deamination of dl-allothreonine in the dog. In preliminary studies, not to be reported in this paper, we have indications that dl-threonine is not deaminized as rapidly as glycine, alanine, or some of the other acids.

The second procedure employed to determine carbohydrate formation was a method used by Butts, Dunn and Hallman (7). Male rats 125 to

185 grams in weight were fed 15.0 grams of sodium butyrate (as acetone) per square meter of body surface per day. The second, third and fourth days, the experimental animals were fed 15.4 grams of dl-threonine or dl-allothreonine per square meter per day and acetone body excretion was determined by the method of Van Slyke. Total urinary nitrogen excretion was determined by the Kjeldahl method. The results are given in table 2.

There was a significant reduction in acetone body excretion on feeding the amino acids with the sodium butyrate. This reduction in ketonuria indicates the potentialities of these amino acids for carbohydrate formation.

Though as previously mentioned, Snyder and Corley (5) concluded that dl-allothreonine was not significantly deaminized, when injected into the dog, this apparent contradiction with the present study is probably not as real as it seems. There appears to be a species difference in the metab-

TABLE 2

Acetone body excretion by male rats receiving 15.0 grams of butyric acid (calculated as acetone) per square meter per day together with dl-threonine or dl-allothreonine

NUMBER OF ANIMALS IN GROUP	AMINO ACID FED	AMOUNT OF ACETONE BODY EXCRETION MGM. PER 100 GRAMS OF RAT		
		1st day	2nd day	3rd day
3	None	122	161	165
4	dl-Threonine	71	85	74
4	dl-Allothreonine	77	84	84

olism of dl-allothreonine between the dog and the rat, though it might be pointed out that Snyder and Corley's figures do show slight increases in ammonia nitrogen or in urea and ammonia nitrogen excretion after injection of the dl-allothreonine. Our study obviously does not differentiate between the action of the optical isomers. It might be mentioned, however, that in a preliminary study with dogs there was no evidence, from a polariscopic examination of the urine, of a preferential utilization of either optical isomer of either the threonine or of the allothreonine.

SUMMARY

The feeding of either dl-threonine or dl-allothreonine resulted in definite increases in liver glycogen. Similarly, both substances decreased the ketonuria resulting from feeding butyric acid. This indicates that both dl-threonine and dl-allothreonine may be transformed to carbohydrate in the rat.

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THE EFFECT OF BILE ON THE QUANTITY AND DIGESTIVE ACTIVITY OF SMALL INTESTINAL JUICE[1]

H. F. HANEY, W. C. ROLEY AND P. A. COLE

From the Department of Physiology, University of Oregon Medical School, Portland

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Although the control of the secretion of the small intestine has received little attention in the literature, certain facts regarding it have been quite well established. Extracts of the intestinal mucosa have been reported to contain a hormone, enterokrinin, which is capable of exciting the glands of both the innervated and the denervated small intestinal loop in the dog (5). Numerous observations indicate that mechanical stimulation as produced by distention serves as a potent stimulus to intestinal secretion (3, 4). The oral administration of such foods as eggs and milk, glucose solutions, and solutions of iron salts (7) and of peptone (6) have been found to result in an increased rate of secretion. On the other hand, observations of the effect of local application or presence of carbohydrate solutions, of tenth normal and twentieth normal hydrochloric acid and of bile salts failed to furnish evidence of any specific stimulating effect (7).

In the course of a study which demonstrated that propulsive motility of loops of the small intestine is increased following the local application of bile (1), increased amounts of intestinal juice were observed to flow from the fistula, apparently in response to the same stimulating influence (2). Five explanations for this increased flow of juice seemed possible. First, it might be produced as a result of the emptying action of increased propulsive movements on juice already present in the loop. Secondly, a reflex effect of a stimulating action of bile on visceral receptors would possibly explain the result. Thirdly, the liberation of a hormone comparable to Nassett's enterokrinin in response to a local action of bile might be postulated. Fourthly, although it was guarded against, the possibility remains that mechanical stimulation incident to the introduction of the test solutions was the cause of the increased secretion. Fifthly, the increased flow of juice might be caused by a local stimulating action of bile.

In order to obtain further evidence regarding a possible stimulating effect of bile on the glands of the small intestine, and to find whether the

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increased flow of juice was accompanied by an increase in enzyme output, the following experiments have been performed.

METHOD. One or two Thiry loops of about 8 to 10 cm. in length, and with distal ends to the exterior, were prepared in 7 dogs from the upper jejunum as near as convenient to the ligament of Treitz. Three groups of animals were used. In the first group only one Thiry loop was prepared; in the second, two innervated Thiry loops; and in the third, one innervated and one denervated loop.

After a training period of a few weeks during which the animal was taught to lie quietly throughout the period of a test, the following procedure was used.

The loop was thoroughly washed with isotonic saline buffered at pH 6.8 and at body temperature. The solution was introduced by means of a small catheter and syringe. Final content of the loop was carefully aspirated.

A 4 cc. portion of the solution to be tested was then introduced into the depths of the loop at 10 minute intervals until 6 applications had been made. Care was taken to produce as little mechanical stimulation as possible. The fluid draining from the fistula during the one hour collection period was collected in a small vessel held in such a position that no loss of fluid occurred except in rare instances, the results being discarded in the latter case. The constant presence of an attendant throughout the period of collection is essential.

At the end of the one hour collection period the loop was again washed with buffered isotonic saline, its final contents were aspirated, and the washings were pooled with the collection.

The enzymic activity of the fluid collected was determined for its ability to digest sucrose. Digestion mixtures were made up of one-fourth of one hour's collection from a loop, 25 cc. of 2 per cent sucrose buffered with phosphate buffer at pH 6.8, one-half cubic centimeter of toluene, one-half cubic centimeter of bile salt solution, and sufficient of the isotonic buffered saline to bring the volume to 50 cc. Two digestion mixtures were prepared for each specimen collected, one containing boiled and the other unboiled juice.

The glucose equivalent of the digests after 24 hours of incubation at 38°C. was determined on protein-free filtrates by means of the Shafer-Somogyi method. Data are given in terms of the difference in glucose equivalent of boiled and unboiled specimens; i.e., the net glucose equivalent.

Each experiment was preceded by a fast of 24 hours and included at least one control collection period of a duration of one hour and an experimental period of equal duration. During the control period saline solution buffered with phosphate at pH 6.8 was used and during the experimental

period either dogs' gall bladder bile or a 7½ per cent bile salt[2] solution similarly buffered to the same pH. All solutions were made approximately isotonic with blood as judged by determinations of the depression of the freezing point.

Additional controls with saline are described in 1, *d* of Results.

TABLE 1

Dogs having a single innervated Thiry loop

DOG NUMBER	TEST HOUR	SOLUTION USED*	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.	DOG NUMBER	TEST HOUR	SOLUTION USED*	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.
			cc.					cc.	
1	1st	Saline	0	497	3	1st	Saline	0	342
	2nd	Saline	−4	165		2nd	Bile	+7	510
3	1st	Saline	+1	384	4	1st	Saline	+1	540
	2nd	Saline	0	240		2nd	Bile	+9½	754
4	1st	Saline	0	434	4	1st	Saline	+½	472
	2nd	Saline	+1½	282		2nd	Bile	+7	787
1	1st	Saline	+1½	290	1	1st	Na taur.	+9	477
	2nd	Na taur.	+6	356		2nd	Saline	+3½	342
	3rd	Saline	+3½	181		3rd	Na taur.	+5½	461
2	1st	Saline	+½	398	2	1st	Na taur.	+1	358
	2nd	Na taur.	+2	484		2nd	Saline	−1½	65
	3rd	Saline	−½	201		3rd	Na taur.	0	293
4	1st	Saline	−½	673	3	1st	Na taur.	+7	683
	2nd	Na taur.	+10	827		2nd	Saline	+1½	227
	3rd	Saline	+1½	358		3rd	Na taur.	+7	564
4	1st	Saline	+1½	547	1	1st	Na glyco.	+6	423
	2nd	Na glyco.	+4	750		2nd	Saline	+1	168
5	1st	Saline	+2½	426		3rd	Na glyco.	+4	429
	2nd	Na glyco.	+4	606	2	1st	Na glyco.	+2½	181
5	1st	Saline	+1	411		2nd	Saline	+1	86
	2nd	Na glyco.	+6½	515		3rd	Na glyco.	+1½	146
					3	1st	Na glyco.	+6½	819
						2nd	Saline	+2½	233
						3rd	Na glyco.	+6½	535

* Na taur. = sodium taurocholate solution; Na glyco. = sodium glycocholate solution.

RESULTS: 1. Five dogs with a single innervated Thiry loop. *a*. The effect of application of dogs' gall bladder bile. In 4 of 5 experiments on one dog and in all of the 3 experiments on a second dog marked increases in net glucose equivalent were found in the digests representing the period during which bile was used, as compared with the control period. The

[2] Pfanstiehl.

increases ranged from 22 per cent to 66 per cent. See data of table 1. Changes in volume during the control period ranged from 0 to +2 cc. and during the period of bile application from +4 cc. to +12 cc.

b. The effect of sodium taurocholate solution. In all of 18 experiments on 4 dogs there was a marked increase in net glucose equivalent in the digests representing the bile salt solution as compared with those of the control. These increases ranged from 21 per cent to 450 per cent above the corresponding controls. Volume changes for the controls ranged from $-1\frac{1}{2}$ cc. to +4 cc., and for the period during which the bile salt was applied from +1 cc. to +12 cc. Thirteen of 18 tests using the bile salt solution showed increases in volume of more than 5 cc. over that of the controls. Representative data are given in table 1.

c. The effect of sodium glycocholate solution. In 11 experiments on 4 dogs all except one showed an increase in net glucose equivalent above 37 per cent, the increase ranging up to 763 per cent. Volume changes for the controls ranged from −3 cc. to +5 cc. and for the bile salt solution from $+1\frac{1}{2}$ cc. to $+9\frac{1}{2}$ cc. See table 1 for representative data.

d. The effect of repetition of the control solution, buffered isotonic saline, during a second consecutive hour. In 9 of 14 experiments on 4 dogs there was less digestion during the second hour than during the first; while of the other 5 experiments, 2 showed no change and 3 showed slight increases of 14 per cent, 18 per cent and 14 per cent, respectively. Representative data are to be found in table 1. Volume changes ranged from 0 to +4 cc. during the first hour and from −4 cc. to +3 cc. during the second hour.

2. In 2 dogs with two innervated Thiry loops each, the control solution was used during two consecutive hours in loop I while in loop II the control solution was used during the first hour and bile or one of the bile salt solutions during the second hour. Any reflex effect of the experimental solutions should then be detectable in the response of loop I. In loop II the response was similar to that observed in other experiments with the same test solution. In 11 of 12 experiments on 2 dogs the increases in net glucose equivalent ranged from 12 per cent to 168 per cent, and one showed a decrease of 2.5 per cent. Nine of the 12 showed an increase of 25 per cent or over, and 6 an increase of 34 per cent or over. In the same experiments loop I behaved in response to the control solution as though it were independent of reflex effects from loop II. Thus in 10 of the 12 experiments there was a reduction of net glucose equivalent in the specimen of the second hour as compared to the first, while in only 2 was there a slight increase, in one case of 17 per cent and in the other of 18 per cent. Similarly the volume changes failed to indicate any effect of introduction of bile or bile salt solutions into one loop on the secretion of the other. Examples of these experiments are given in table 2.

3. In 2 dogs with loops that had been denervated by mesenteric stripping and application of phenol to the vessels, the response to sodium taurocholate solution was fully as great as in the case of normal dogs. Thus in 6 of 7 experiments on 2 dogs the increases in net glucose equivalent ranged from 42 per cent to 134 per cent and in the seventh 10 per cent.

TABLE 2

Dogs with two Thiry loops. Effect of introduction of test solution into one loop on juice from second loop

DOG NUMBER	LOOP NUMBER	TEST HOUR	SOLUTION USED	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.
				cc.	
4	I	1st	Saline	−2	362
		2nd	Saline	−2	247
	II	1st	Saline	+2	393
		2nd	Bile	+5	527
5	I	1st	Saline	0	255
		2nd	Saline	+1	114
	II	1st	Saline	+½	256
		2nd	Na taur.*	+8	688

* Sodium taurocholate solution.

TABLE 3

Dogs with denervated Thiry loops

DOG NUMBER	TEST HOUR	SOLUTION USED*	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.	DOG NUMBER	TEST HOUR	SOLUTION USED*	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.
			cc.					cc.	
6	1st	Saline	+1½	395	7	1st	Saline	−3	291
	2nd	Na taur.	+9½	925		2nd	Na glyco.	+3½	621
6	1st	Saline	−1	184	6	1st	Saline	+2	339
	2nd	Na taur.	+4½	369		2nd	Na glyco.	+4	540
7	1st	Saline	−2	149	7	1st	Saline	+2	625
	2nd	Na taur.	+4	289		2nd	Na taur.	+6	885

* Na taur. = sodium taurocholate solution; Na glyco. = sodium glycocholate solution.

Volume changes in the saline controls ranged from −3 cc. to +2 cc. and in those using bile salt solution from +2 cc. to +9½ cc. It would seem then that the effect of locally applied bile and bile salts on the volume and digestive activity of the juice collected from loops of the small intestine does not depend on their extrinsic nerve supply. Representative data are presented in table 3.

DISCUSSION. As indicated in the recent report of Wright et al. (9), there has been some question as to whether intestinal juice owes its disaccharide-splitting power to exo-enzymes secreted by gland cells or to endo-enzymes present in suspended mucosal cells. Pierce et al. (8) found that centrifuged intestinal juice has a more feeble digestive action on sucrose and other substrates than does uncentrifuged juice. They attributed the difference to the presence in the latter of a suspension of amorphous material and a few mucosal cells, and suggested that the enzymes might be adsorbed by this material.

In the present work the collected juice was shaken with glass beads as suggested by Pierce et al. (8) before the sample to be incubated was withdrawn from the collecting vessel. Since the amount of mechanical stimulation incident to the introduction of the test solution and various control solutions was in all cases approximately equal, it would seem that the shedding of increased numbers of mucosal cells containing invertase would not account for the increases in digestive action found. While the possibility that local action of bile or bile salts might conceivably result in the shedding of mucosal cells must be considered, such an effect would in no sense detract from the importance of the action of bile in causing increase in volume and in disaccharide-splitting action of intestinal juice.

The fact that the increases in secretion were demonstrated in previously washed loops seems to rule out the possibility that increase in outflow of juice observed in previous experiments is caused by increased propulsive movements.

The possibility of a reflex mechanism which would account for the increase in secretion following application of bile is ruled out by the good responses obtained from denervated loops.

The failure to obtain an increase in secretion of a second Thiry loop while the first one was being stimulated by locally applied bile salt solution argues against a hormonal as well as a reflex activation of the glands in response to bile salt solution.

The fact that mechanical stimulation incident to the introduction of test solutions was equal in the case of control and experimental solutions would seem to rule out this mode of stimulation as a possible causative agent. It may still be claimed however that the increase in propulsive movements which follows application of bile salts possibly might result in mechanical stimulation of the glands. It is questionable however whether these movements are sufficiently intense to provoke such a stimulation.

A local stimulating action of bile salts on the glands of the small intestine readily explains the increased secretion observed in these experiments. The relative importance of this action in the normal physiology of the small intestine is not entirely apparent. It would appear that bile salts may well serve as a part of the normal mechanism for stimulation of the

glands of the small intestine and that through this means they aid in digestion. In the absence of bile salts the rate of secretion of the small intestinal glands may be expected to be considerably less than during their presence.

SUMMARY

Gall bladder bile of the dog was applied locally to the mucosa of Thiry loops of the jejunum of dogs, and the outflow of fluid from the loop was collected. During a given collection period when bile was applied, the specimen obtained in unit time was greater in volume and in its ability to digest sucrose than were similar specimens collected during application of isotonic saline buffered at pH 6.8. Local applications of both sodium glycocholate and sodium taurocholate in 7.5 per cent solution adjusted to isotonicity by addition of sodium chloride and buffered with phosphate at pH 6.8 produced results essentially the same as those obtained with bile.

Application of bile or bile salt solution to the mucosa of one of two innervated Thiry loops in a given dog failed to influence the secretion of the other loop.

Denervated Thiry loops responded fully as well as, but not better than, those whose innervation remained intact.

It is concluded that local application of bile to the mucosa of jejunal loops in the dog results in increased volume and digestive activity of the intestinal juice collected in unit time. In the light of the data presented a local stimulating action of bile salts on the glands of the jejunal mucosa best explains this result.

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VITAMIN E DEFICIENCY IN THE MOUSE[1]

W. L. BRYAN AND K. E. MASON

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee

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Proof of the existence of vitamin E is based primarily upon the characteristic impairment of reproduction observed in rats reared on highly purified diets deficient in this substance but adequate in all other respects. Evidence concerning the need of this vitamin for reproductive function in other vertebrates is either of circumstantial nature or is based upon the use of diets of natural foodstuffs subjected to chemical treatment (ferric chloride in ether) in an effort to destroy or inactivate the vitamin E present. Considerable evidence has been obtained in this laboratory (unpublished studies) indicating that the latter objective is not always attained by this procedure. Attempts to induce a state of vitamin E depletion in the mouse, the only other laboratory animal which readily consumes the concentrated and purified type of diet such as used for rats, appear to be limited to the early studies of Beard (1926). Since the diet used in the latter studies contained 20 per cent of hydrogenated cottonseed oil (Crisco), which has since been shown to constitute a very good source of vitamin E, it is evident that the sterility observed in mice of both sexes cannot be attributed to an inadequacy of this vitamin and must be explained on some other basis.

The sterility noted by Beard in female mice was characterized by a marked decrease in the number of litters produced in comparison with mice maintained on the stock diet. Pregnancy was based upon weight changes in the animal and was not verified by examination of the vaginal smears. Furthermore, the sterile mice were not examined for gross or histological evidence of fetal resorption. At the time these studies were made little was known concerning the nature of this latter process which has since been so thoroughly studied in the rat (Evans and Burr, 1927). Sterility in the males was characterized by infrequency of fertile matings when placed with normal females, and by degenerative changes observed in the germinal epithelium of the testis. It should be stated that one of us (K. E. M.) had the privilege of examining histological preparations of

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the testes from these mice and, at that time, was of the opinion that the injury observed resembled that encountered in the vitamin E deficient rat. However, the evidence was not conclusive on this point and was not supported by attempts to repair the testicular damage which, in the light of later knowledge, would have offered a ready means of determining whether lack of vitamin E was the limiting factor. It was felt that a reinvestigation of reproductive functions in mice would constitute a definite contribution to the limited knowledge concerning vitamin E requirements of different animal species.

Experimental. Mice of the breeding colony were maintained on a diet of commercial dog biscuit known to afford about three times the minimal daily requirement of vitamin E for the rat. At the 10th day of lactation mothers and their progeny were transferred to the vitamin E deficient diet, upon which the young mice were maintained after weaning at the 21st day of age. The mice were housed in metal cages with wood shavings, except for one series of males reared in wire cages with raised screen floors such as employed in our studies with rats. The composition of the deficient diet used, and data concerning the effectiveness of this same dietary procedure in inducing early sterility in male and female rats, are presented in the following report (Mason, 1940). The experimental female mice, mated with normal stock males as soon as sexual maturity was attained, were successfully inseminated at an average age of 70 days. Vaginal smears were made daily prior to mating and at frequent intervals during pregnancy. Testes and epididymides, obtained from experimental males by operation or at autopsy, were routinely fixed and sectioned for histological examination.

Results. Our experiences in producing, and in preventing, sterility in mice under these conditions are summarized in table 1. It will be noted that the females consistently resorbed during their first pregnancy (groups 1 to 3) and that the sterility could be prevented by administration of fresh raw wheat germ, or wheat germ oil, during early stages of the second pregnancy (group 3). First pregnancy resorptions were readily prevented by administering a concentrate from wheat germ oil (group 4) and synthetic alpha tocopherol (group 5) during the first 10 days of pregnancy. Female mice reared on a modification of the E-deficient diet, in which 5 per cent of the lard was replaced by an equivalent amount of hydrogenated cottonseed oil (Crisco), representing one-fourth the concentration of the latter in the diet used by Beard, permitted delivery and lactation of normal litters (group 6). It is of interest that the minimal amount of wheat germ oil concentrate, and of synthetic alpha tocopherol, required to prevent resorption in mice was approximately one half that required by female rats (table 1, footnotes).

When mice of the breeding stock were maintained on diets containing

relatively large amounts of vitamin E, and the litters given access to this diet during lactation, storage of the vitamin in the newly weaned females

TABLE 1

Reproductive behavior of mice of both sexes reared and maintained on a diet deficient in vitamin E

GROUP	NUMBER AND SEX OF MICE	REPRODUCTIVE PERFORMANCE
1	6 females	Resorbed during first and second pregnancies. The second resorption verified by autopsy on the 16th day
2	12 females	Resorbed during first pregnancy. Verified by autopsy on the 16th day
3	14 females	Resorbed during first pregnancy. Fertility restored by feeding wheat germ, or wheat germ oil, during early stages of the second pregnancy
4	14 females	Fed concentrate from wheat germ oil at first pregnancy:* 0 mgm.—3 neg. 15 mgm.—2 neg. and 3 pos. 20 mgm.—3 pos. 25 mgm.—3 pos.
5	20 females	Fed synthetic alpha tocopherol at first pregnancy:† 0 mgm.—5 neg. 0.125 mgm.—2 neg. 0.25 mgm.—1 neg. and 1 pos. 0.35 mgm.—3 pos. 0.50 mgm.—3 pos. 0.70 mgm.—3 pos. 1.0 mgm.—2 pos.
6	7 females	5% of hydrogenated cottonseed oil (Crisco) included in -E diet. 1st pregnancy resulted in delivery of normal litters, successfully suckled
7	40 males	18 on experiment for 100–200 days 10 on experiment for 200–300 days 12 on experiment for 300–400 days Testis and epididymis histologically normal in all cases

* Fed in a single dose on the 4th day of pregnancy, as an ether solution evaporated on a small amount of dextrin. Thirty milligrams of this concentrate represented the mean fertility dose (i.e., that giving a positive response in 50 per cent of bio-assay tests) for female rats.

† Supplied through the courtesy of Merck and Co., Inc., Rahway, N. J. The tocopherol was administered orally as an olive oil solution, the total dose being distributed over the first 10 days of pregnancy. Bio-assay tests reported elsewhere (Mason, 1940; table 4) indicated that 0.7 mgm. of this tocopherol represented close to the mean fertility dose for female rats. In groups 4 and 5, a positive response implied either the presence of two or more living fetuses at the 16th day of pregnancy or the delivery of litters at full term.

was sufficient to permit one or two pregnancies before the occurrence of a typical resorption gestation. This "first litter fertility" occurs in rats under similar conditions. The histopathological changes observed in the

resorption sites resembled in every way those encountered in the E-deficient rat. The same was true of implantation sites containing dead fetuses, several of which were encountered in mice given critically low doses of vitamin E and autopsied during the last week of pregnancy. This phenomenon of late fetal death, which we have frequently observed in rats used for bio-assay tests and autopsied at the 16th day of pregnancy, will be discussed more fully in a later communication. The neuromuscular disorder so frequently seen during late stages of lactation in the progeny of female rats possessing a critically low storage of vitamin E was not observed in any of our suckling mice. It is possible that the level of vitamin E depletion necessary for the appearance of this phenomenon was not attained in our experiments. On the basis of the evidence presented above, it can be assumed that the female mouse responds qualitatively to vitamin E depletion and to vitamin E therapy in the same manner as does the female rat, and manifests the same histopathological alterations in the fetus and fetal membranes.

On the other hand, our efforts to induce sterility in male mice have met with complete failure. Forty male mice, mostly littermate brothers of the females described above, were prepared and reared in exactly the same manner. After 100 to 400 days of experimental feeding (group 7; table 1) the testes and epididymides from each mouse were examined histologically. In no instance was there any evidence of testicular degeneration. These findings are quite contrary to our experiences with male rats in which the same experimental procedure consistently produces histopathological changes in the seminiferous epithelium after an experimental period of 35 to 50 days (Mason and Bryan, 1940; Mason, 1940).

Discussion. It seems difficult to escape the conclusion that the vitamin E requirements of the male mouse, if the latter can be shown to be dependent upon an extraneous source of vitamin E, must be very much less than those of the female mouse. We were at one time of the opinion that such a sex difference in vitamin E requirements existed in the case of the rat, but have since been able to demonstrate that the minimal daily intake necessary to maintain reproductive functions in the male and female rat is essentially the same and have attributed this apparent sex difference in E-requirements to age differences in the manifestation of urgent needs for the vitamin in the two sexes (Mason, 1940). These qualitative differences are of such a nature that sterility should be more readily produced in males than in females if the presence of traces of vitamin E in the experimental diet were in any way related to the striking differences observed in the response of male and female mice to deprivation of vitamin E. Unfortunately, the experimental data presented in this report offer no adequate explanation for the latter phenomenon.

SUMMARY

1. Female mice reared on a purified vitamin E deficient diet exhibited resorption of the fetuses which, on the basis of gross and histological examination of the placental sites, was indistinguishable from that observed in the vitamin E deficient rat. Sterility was prevented by feeding natural sources of vitamin E or synthetic alpha tocopherol.

2. The same dietary procedure, which also induces sterility in rats of both sexes at the onset of sexual maturity, failed to produce testicular injury in male mice after an experimental feeding period of 400 days.

3. Good reproduction observed in female mice when 5 per cent of the lard in the deficient diet was replaced by an equivalent amount of hydrogenated cottonseed oil, suggests that the previously reported observations concerning sterility in mice due to inadequate vitamin E (Beard, 1926) are subject to some other interpretation.

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MINIMAL REQUIREMENTS OF MALE AND FEMALE RATS FOR VITAMIN E[1]

KARL E. MASON

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee

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It has been suggested that vitamin E may be bipartite in nature, consisting of separate factors responsible for fertility in the two sexes of the rat (Grijns and Dingemanse, 1933; Grijns, 1939). These conclusions were based upon the observation that concentrates obtained from wheat germ oil, separable on the basis of their solubility in alcohol and benzol, differed in their ability to prevent sterility in male and female rats. Grijns (1939) also observed that certain of his experimental diets caused sterility in males but not in females, while other diets induced sterility in females but not in males, which he interpreted as confirming the existence of separate anti-sterility factors for the two sexes. The observation that the presence of 8 per cent butter (Geller and Schuster, 1934), or of 20 per cent tapioca starch (Martino, 1934), in diets otherwise deficient in vitamin E causes sterility in female rats but not in males has also prompted the suggestion referred to above. Various other investigators have noted that certain diets presumably low in vitamin E sometimes prove more effective in causing sterility in one sex than in the other, the preponderance of opinion indicating that the vitamin E requirements of the male rat are considerably greater than those of the female.

Some experimental evidence obtained in this laboratory has been suggestive of a slightly greater requirement for males than for females. Certain diets treated with ferric chloride in ether have produced an irreversible testis injury in male rats indistinguishable from that of E-deficiency but have permitted continued reproduction in littermate sister rats, while other diets treated in a similar manner have induced sterility in both sexes. Furthermore, purified E-deficient diets containing 20 per cent of winter butter have caused early testis injury in males but no sterility in females, although the same proportion of summer butter in the diet has permitted continued fertility in both sexes (Mason and Bryan, unpublished studies). On the other hand, it has been noted that single doses of wheat germ oil

[1] This investigation was aided by a grant to Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

concentrates administered to newly weaned rats reared on an E-deficient diet may postpone the onset of testis degeneration beyond the 100th day of life and yet prove ineffective in preventing resorptions in females mated at an average age of 66 days (Mason and Bryan, 1940). The latter observations led us to postulate that the vitamin E requirements of male rats must be considerably less than those of females (Mason, 1939). In accord with this assumption was our inability to induce testis injury in male mice under experimental conditions which readily produced first pregnancy resorptions in females (Bryan and Mason, 1940), suggestive of a much greater difference in sex requirements for vitamin E in this species of rodent. However, we have acquired considerable data indicating that the reported observations on rats suggesting the existence of more than one anti-sterility factor, or marked sex differences in requirements for a single factor, may be explained on the basis of differences in the age of male and female rats at which vitamin E, in the form of initial storage or subsequent acquisition of the vitamin, becomes effective in the prevention of sterility. This report presents experimental evidence concerning such qualitative time differences in vitamin E needs, together with data relating to quantitative requirements of the male and female rat for vitamin E.

EXPERIMENTAL. Rats of the breeding colony were reared and maintained on a stock diet of commercial dog biscuit affording approximately three times the minimal daily requirement of vitamin E. During the last week of the 21 day lactation period the mothers and their litters were transferred to the vitamin E deficient diet. The latter, which was made up at least twice weekly, was composed of commercial casein (20 per cent), cornstarch (48 per cent), lard (18 per cent), salts (4 per cent), brewer's yeast (8 per cent) and cod liver oil (2 per cent). The progeny, whose storage of vitamin E was thus restricted to the limited amount available through placental and mammary transfer, were maintained on the E-deficient diet after weaning. It has been shown that these rats, referred to as our "standardized" rats, are uniformly and critically depleted of vitamin E from the beginning of life, testicular degeneration consistently appearing within 35 to 50 days after weaning (average, 40 days) in the males and fetal resorption invariably occurring in females experiencing their first pregnancy after a similar experimental period (Mason and Bryan, 1938, 1940). This early attainment of sexual maturity in female rats, which are mated at body weights of 150 to 160 grams and usually conceive after an average experimental period of 36 days, is attributed to three factors: 1, restriction of litters to 6 rats; 2, the experimental use of only those which exceed 40 grams in body weight at the 21st day of life, and 3, the improved lactation performance of the mother and stimulated growth in the suckling offspring following their transfer to the concentrated diet.

A. *Qualitative differences in sex requirements for vitamin E.* Despite

the occurrence of testicular degeneration in standardized males in close relation to the onset of sexual maturity, and evidence relative to the severity of vitamin E depletion in these animals from the beginning of life, histopathological changes have not been demonstrable in the germinal epithelium prior to puberty. Since earlier studies (Mason, 1926) had shown that a profound and irreversible physiological disturbance of the germinal epithelium in the E-deficient rat precedes by some days the first appearance of histological damage, it seemed of interest to determine whether the sexually immature testis was affected in like manner. One testis was removed by operation from standardized male rats at intervals of 20 to 40 days on the E-deficient diet (group B, table 1). The operated rats were then given an oral dose of 1 gram of wheat germ oil, transferred to a diet of fresh raw wheat germ, and autopsied at the 60th day of experiment. Representative littermates transferred to the stock diet after weaning, but operated upon and autopsied after similar periods, served as controls (group A, table 1). Histological sections from two portions of each testis, and from the head and tail of the epididymis, were studied microscopically. The data obtained demonstrated that ability of the testis to respond to vitamin E therapy was retained by all rats at the 20th day, was lost by about one half at the 25th day, and was absent in all rats at the 30th day. Similar results were obtained 18 months later in a series of experiments in which the preliminary operative procedure was eliminated (group C, table 1).

During the first 30 days after weaning there were no demonstrable differences in either the growth or the histological state of the seminiferous epithelium of testes from control and experimental rats. Testes removed at the 20th day showed an immature epithelium and the ducts of the epididymis were either empty or contained a few sloughed germ cells. By the 25th day mature spermatozoa were appearing in many of the seminiferous tubules but very few had reached the ducts of the caput epididymis. At the 30th day spermatogenic activity in the testis compared favorably with that seen in the mature testis, although most seminiferous tubules had not attained adult dimensions. The ducts of the epididymis were moderately distended with spermatozoa, and scattered germ cells sloughed from the germinal epithelium of the testis were usually encountered in the more distal ducts of the organ.[2] Not until the 35th and 40th days did the E-defi-

[2] Small numbers of incompletely differentiated cells are normally sloughed from the germinal epithelium and appear in the ducts of the epididymis prior to the beginning of spermatogenic activity. Sometimes the first crop of spermatozoa which appear in the epididymal ducts, intermingled with these cells, also appear atypical in structure. Consequently, it is sometimes difficult to discriminate between these latter changes, which are of normal occurrence, and similar changes which immediately precede and accompany the first demonstrable evidence of E-deficiency degeneration.

cient rats begin to show histological evidence of testicular degeneration (in slightly more than half the animals in the two groups) although irreparable damage had been induced in the majority of rats at the 25th day of feeding.

TABLE 1

Showing the stage of experimental feeding at which the testes of standardized male rats lose their ability to respond to vitamin E therapy

DIET AFTER WEANING, DAYS	NO. OF RATS	BODY WT. IN GM., AVER.	TESTES REMOVED AT OPERATION			DIET AFTER WEANING, DAYS	NO. OF RATS	BODY WT. IN GM., AVER.	TESTES REMOVED AT AUTOPSY		
			Wt. in gm., aver.	Per cent normal,* aver.	Histology†				Wt. in gm., aver.	Per cent normal,* aver.	Histology†
Group A											
S20	4	116	0.585	80	Normal	S60	4	251	1.46	114	Normal
S25	4	133	0.806	98	Normal	S60	4	237	1.43	120	Normal
S30	6	155	1.063	113	Normal	S60	4	234	1.52	128	Normal
Group B											
−E20	8	114	0.670	92	Normal	−E20+E40	8	244	1.54	127	Normal
−E25	11	148	0.887	101	Normal	−E25+E35	4	253	1.50	124	Normal
							7	251	0.84	68	2 (P-P); 5 (4-5)
−E30	10	188	0.975	113	Normal	−E30+E30	6	247	0.62	51	6 (4-5)
−E35	7	200	1.18	109	4 (N); 3 (N?-1)	−E35+E25	7	251	0.79	66	2 (P-P); 2 (2-4); 3 (4-5)
−E40	8	221	0.97	90	3 (N); 2 (N?-1); 2 (1); 1 (2-3)	−E40+E20	4	233	0.83	71	4 (4-5)
Group C											
						−E20+E40	5	261	3.02 (2)	128	Normal
						−E25+E35	5	257	2.99 (2)	120	Normal
							7	260	2.21 (2)	88	7 (P-P)
						−E30+E30	6	257	1.68 (2)	66	1 (N); 1 (P-P); 4 (4-5)

* Based on the figures of Donaldson (1924) for normal testis weight in relation to body weight.

† The stage, or stages, of degeneration predominating in the testes are indicated in parentheses, and are based upon the arbitrary divisions of the degenerative process described in an earlier report (Mason, 1926). The designation P-P refers to the condition of "partial prevention," characterized by the occurrence of completely degenerated seminiferous tubules intermingled with variable numbers of normal tubules in which the germinal epithelium, having suffered no irreparable injury at the moment vitamin E therapy became effective, was protected against degenerative changes.

These observations indicate that standardized male rats manifest a critical need for vitamin E within 20 to 25 days after weaning (41 to 46 days of age) in order to prevent an irreversible physiological disturbance in the germinal epithelium which precedes, by at least 10 days, the appearance of histopathological alterations. On the other hand, the vitamin E needs of the female rat for reproductive success are of a distinctly different

order. These needs arise only after conception occurs, can be satisfied at any time during the first week of pregnancy, and recur periodically throughout the reproductive period. Under optimum conditions for rapid growth and early sexual maturity, conception in females usually follows by at least two or three weeks the time at which males manifest their critical needs for vitamin E. There is also some evidence (Mason and Bryan, 1940) that the female requires during the first week of pregnancy a relatively greater concentration of vitamin E to prevent fetal resorption than would be required by males at their critical stage in order to postpone the onset of testis degeneration for a period comparable to the life of the developing fetus. It is thus apparent that if rats of both sexes possess the same initial storage of vitamin E at weaning, and are subsequently reared on an E-deficient diet, the males will be able to take better advantage of this storage than the females in whom much of the original supply may be dissipated by general metabolic processes before it is required for reproductive functions. This is well illustrated by observations presented elsewhere (Mason and Bryan, 1940) demonstrating that one can administer to newly weaned standardized rats of both sexes amounts of vitamin E sufficient to postpone the onset of testicular degeneration beyond the 100th day of life and yet fail to prevent resorption in females inseminated at an average age of 66 days.

On the basis of what has just been said it is conceivable that under experimental conditions characterized by an appreciable storage of vitamin E in newly weaned rats, with or without the presence of traces of E in the diet, the males might be able to maintain testis normality up to and beyond a time when sterility was demonstrable in females. On the other hand, under conditions characterized by a negligible storage of E in newly weaned rats subsequently reared on a diet containing traces of the vitamin, the males might not acquire sufficient vitamin by the 40th to 45th day of age to ward off testicular damage although females might eventually store enough to permit the completion of gestation. The latter situation would naturally be accentuated by any dietary increment in vitamin E subsequent to the period at which the needs of the male become critical. Failure to realize these qualitative differences in vitamin E requirements for the rat may explain why investigators have been led to suggest the existence of separate factors, or the need for widely different amounts of the same factor, for the prevention of sterility in the two sexes. In an effort to determine whether male and female rats actually differ quantitatively in their requirements for vitamin E, the following investigations were undertaken.

B. *Quantitative requirements for vitamin E.* Standardized male and female rats were given small daily doses of vitamin E in the form of a concentrate from wheat germ oil and synthetic alpha tocopherol, diluted

with pure olive oil such that each daily dose involved the administration of 0.05 to 0.20 cc. of solution, fed orally by means of small tuberculin syringes. The absence of vitamin E in olive oil had been demonstrated by the sterility consistently observed in standardized rats reared on an E-deficient diet containing 20 per cent of this oil as the fat constituent of the ration. The stock solutions and the small portions removed as needed for feeding were kept at 0°C. Daily dosage was begun on the 10th day after weaning, at which time the rats had an average body weight of 80 grams, and was continued throughout the experimental period except in a few instances as indicated in the summarized data (tables 2 and 3).

Males. Thirty two male rats were given graded doses of the two preparations. In view of extensive data already available in this laboratory concerning the onset of testis injury in the standardized male rats the untreated controls were limited to 6 rats, all of which responded in typical manner. One testis was removed by operation at the time of expected onset of degenerative changes, the other testis being taken at autopsy 5 to 20 days later. A study of stained smears from the testis and from the head and tail of the epididymis, obtained at the time of testis removal, afforded a preliminary analysis of the extent of injury. Representative sections from each testis and epididymis were subsequently examined histologically and the testes classified according to the predominant state or stages of degeneration present (Mason, 1926), the epididymis serving as a useful index of recent events in the seminiferous epithelium. Correlation of these observations with data obtained concerning the time intervals between the different stages of degeneration permitted a relatively accurate estimate of the date of onset of histopathological injury. The results of these studies are summarized in table 2.

Daily doses of 0.0625, 0.125, 0.25, 0.5 and 1 mgm. of the wheat germ oil concentrate effected a significant delay in the onset of testis injury, which became more apparent at increased levels of dosage, but eventually failed to afford protection. Doses of 2 mgm. daily proved adequate throughout the experimental period of 80 days, although cessation of dosage at the 60th day of experiment resulted in the onset of degeneration within two weeks. Daily doses of 4 mgm. afforded adequate protection throughout a 90 day period, even when dosage was discontinued at the 70th day. It appears that a daily intake of 2 mgm. of this concentrate can be regarded as being close to the minimum for maintaining structural integrity of the testis in the standardized male rat.

A daily intake of 0.0375 mgm. of alpha tocopherol effected an average delay of only 11 days in the onset of testis injury, although twice this level of intake (0.070 mgm.) afforded complete protection throughout the experimental period. In the one rat continued to the 90th day, the daily dose was discontinued at the 70th day as a check on the extent of vitamin E

TABLE 2

Showing the effectiveness of a concentrate from wheat germ oil and of alpha tocopherol, administered in daily doses from the 10th day after weaning, upon the onset of testis degeneration in standardized male rats

DAILY DOSE	TOTAL DOSE	TESTIS AND EPIDIDYMIS REMOVED				DAYS ON EXPER. AT ONSET OF TESTIS DEGENERATION (ESTIMATED)	
		At operation		At autopsy			
		Da. on exper.	Stage of degen.	Da. on exper.	Stage of degen.		
Controls							
mgm.	*mgm.*						*aver.*
0	0	50	4–5	55	5	37	40.5
		40	N	50	N?–1	48	
		40	1–2–3	50	4–5	34	
		40	N	50	1	46	
		40	N–N?	50	3–4	39	
		40	N?–1	50	3–4	38	
E-concentrate*							
0.0625	2.8	50	2–4	55	4–5	42	43.0
0.0625	2.8			55	1–5	44	
0.125	5.6	50	4	55	5	40	45.5
0.125	5.6			55	1–2	51	
0.25	11.2	50	1–2–3	55	2–5	44	47.2
0.25	11.2			55	3–4	47	
0.25	11.2	50	N–N?	55	1–2	50	
0.25	11.2	50	N?–1	55	2–3	48	
0.50	25	60	2–4	70	5	52	53.5
0.50	25	55	1	60	2–3	52	
0.50	25	55	N	60	1	57	
0.50	25	55	N?–1	60	2–3	53	
1.0	60	60	N	70	2–3–4	62	56.2
1.0	50	55	N–N?	60	1–2	55	
1.0	50	55	N–N?	60	1–2–3	54	
1.0	60	60	1–2–3	70	4–5	54	
2.0	100	70	3–4	80	5	62‡	
2.0	100	60	N	70	N?–1	68‡	
2.0	100	60	N	80	1–2–3	74‡	
2.0	120	60	N	80	N		
2.0	140	60	N	80	N		

* A molecular distillate of wheat germ oil kindly supplied by the Research Laboratories of General Mills, Inc., Minneapolis, Minnesota.

† Synthetic alpha tocopherol supplied through the courtesy of Merck and Co., Inc., Rahway, N. J.

‡ Daily dose discontinued after the 60th day of experiment.

** Daily dose discontinued after the 70th day of experiment.

TABLE 2—*Concluded*

DAILY DOSE	TOTAL DOSE	TESTIS AND EPIDIDYMIS REMOVED				DAYS ON EXPER. AT ONSET OF TESTIS DEGENERATION (ESTIMATED)	
		At operation		At autopsy			
		Da. on exper.	Stage of degen.	Da. on exper.	Stage of degen.		
E-concentrate*—*Concluded*							
mgm.	*mgm.*						*aver.*
4.0	280	70	N	80	N		
4.0	200	70	N	90	N		
4.0	200	70	N	90	N	**	
Alpha tocopherol†							
0.0375	1.7	50	N	55	N–N?	54	51.5
0.0375	1.7	50	N?	55	1	51	
0.0375	1.7	50	N	55	N?–1	53	
0.0375	1.7	50	N?–1	55	2–3	48	
0.075	4.5	60	N	70	N		
0.075	4.5	60	N	70	N		
0.075	5.25	60	N	80	N		
0.075	6.0	70	N	90	N	**	

storage at that period. It would seem that the minimal daily intake of alpha tocopherol for standardized males lies somewhere between these two levels of dosage.

Females. Forty female rats, similarly fed, were mated with normal males when they had reached body weights of 150 grams or better, and their reproductive activity followed by daily vaginal smears. Conception occurred after an average experimental period of 45 days despite the delay caused by 9 instances of pseudopregnancy. The daily dose of vitamin E was discontinued after the 10th day of pregnancy in all rats. In most instances laparotomy was performed on the 16th day of pregnancy to record the distribution of living fetuses, dead fetuses and resorption sites for subsequent comparison with results obtained at term. At the time of expected delivery the rats were placed upon a coarse wire screen to permit the young to fall out of reach of the mother after delivery. The data obtained are summarized in table 3.

The concentrate from wheat germ oil consistently gave negative responses at a daily intake of 1 mgm. or less, and positive responses at an intake of 4 mgm. or more. A daily intake of 2 mgm. enabled 7 out of 10 rats to show a positive response. The occurrence of dead fetuses in four of these rats also demonstrates the critical character of this level of intake. It is to be noted that the responses elicited by this concentrate, and by the alpha tocopherol, were not always proportional to the total doses administered. This is apparently due to biological variations in animal response

which become particularly evident at critical levels of dosage. Especially significant is the observation that a daily intake of 2 mgm. of the concentrate proved to be close to the minimum for preventing sterility in both

TABLE 3

Reproductive performance of standardized vitamin E deficient rats given small daily doses of 1, a concentrate of wheat germ oil, and 2, synthetic alpha tocopherol, fed orally from the 10th day after weaning to the 10th day of their first gestation period

DAILY DOSE	TOTAL DOSE	UTERUS AT 16TH DAY L. D. R.	DAILY DOSE	TOTAL DOSE	UTERUS AT 16TH DAY L. D. R.
E-concentrate					
mgm.	*mgm.*		*mgm.*	*mgm.*	
0.25	11	0 -0- 9	2.0	72	7 -0- 1 (7)*
0.25	14	0 -0-10	2.0	72	8 -0- 0 (7)
0.25	12	0 -0- 8	2.0	74	7 -2- 0 (5)
0.25	12	0 -0-10	2.0	90	2 -3- 3 (1)
			2.0	82	3 -2- 4 (3)
0.50	23	0 -0-11	2.0	94	6 -0- 2†
0.50	25	0 -0- 7	2.0	90	2 -1- 7†
0.50	22	0 -0-10			
0.50	22	0 -0- 7	4.0	104	6 -0- 3 (4)
			4.0	104	6 -0- 1 (6)
1.0	47	0 -0-11	4.0	144	9 -0- 2†
1.0	49	0 -0- 9	4.0	220	7 -0- 1 (7)
1.0	47	0 -0- 9	4.0	224	8 -0- 5†
1.0	43	0 -0- 9			
			8.0	296	—— (8)
2.0	96	0 -0-11	8.0	424	—— (8)
2.0	98	0 -0-11	8.0	440	—— (8)
2.0	108	0 -0-10			
Alpha tocopherol					
0.0375	1.38	0 -0-10	0.075	2.92	7 -0- 3 (7)
0.0375	1.87	0 -0-10	0.075	3.00	8 -0- 1 (8)
0.0375	2.32	0 -0- 6	0.075	3.00	8 -0- 1 (7)
0.0375	1.95	4 -1- 4 (2)	0.075	3.45	10 -0- 1 (10)
0.0375	2.14	8 -1- 2 (9)	0.075	5.32	8 -0- 2 (8)

* Figures in parentheses indicate the number of offspring delivered at full term.
† Autopsied on 16th day.

sexes. Concurrent bio-assay tests (table 4) indicated that the "mean fertility dose"[3] of this concentrate was slightly in excess of 30 mgm., probably

[3] The term "mean fertility dose," first suggested by Bacharach (1938), denotes the dose capable of giving a positive response in 50 per cent of bio-assay tests It must be kept in mind that the response given at critical levels of dosage may be appreciably modified by differences in the period over which the test dose is administered, as shown by the data in table 4. In this laboratory, administration of the test dose as five equal doses on the 4th to 8th days of pregnancy has proved to be a very satisfactory procedure for general use. The advantages of the 16 day autopsy procedure for evaluating responses will be discussed in a later report.

about 34 mgm., which would represent between fifteen and twenty times the minimal daily dose. It is of interest that this latter dose (2 mgm.) is essentially that calculated by Bacharach (1938) who states "If M be the mean fertility dose found for a substance administered over 10 days to virgin animals about 100 days old, presumably a daily dose of 6M/100 would be quite adequate if given daily from birth, or even from weaning."

The doses of alpha tocopherol selected for daily feeding represented 1/10th and 1/20th of 0.7 mgm. which, according to the recent observations of Bacharach (1939) and the bio-assay data presented in table 4,

TABLE 4

Results of bio-assay tests on the concentrate from wheat germ oil, and the synthetic alpha tocopherol, used in the studies presented in tables 2 and 3

The presence of two or more living fetuses at autopsy on the 16th day of the first pregnancy was taken as a criterion of positive response.

CONCENTRATE FROM WHEAT GERM OIL				ALPHA TOCOPHEROL	
Fed as 5 equal doses on the 4th to 8th days of pregnancy			Fed as 5 equal doses on the 4th day of pregnancy	Fed as 10 equal doses on the 1st to 10th days of pregnancy	Fed as 10 equal doses on the 4th day of pregnancy
Total dose, 20 mgm.	Total dose, 30 mgm.	Total dose, 50 mgm.	Total dose, 50 mgm.	Total dose, 0.7 mgm.	Total dose, 0.7 mgm.
L. D. R.	L. D. R.	L. D. R.	L. D. R.	L. D. R.	L. D. R.
0 -0- 6	0 -0- 5	9 -0- 1	9 -0- 1	10 -0- 0	2 -2- 8
0 -2- 4	0 -1- 0	11 -0- 0	10 -0- 0	8 -0- 1	1 -1- 9
0 -0-10	8 -0- 1	11 -0- 0	12 -0- 0	8 -1- 0	0 -0-11
0 -0- 3	0 -2- 6	8 -0- 1	1 -1-11	7 -0- 0	0 -0-10
0 -0- 8	1 -1- 5	9 -0- 0	3 -0- 6	7 -0- 1	0 -5- 5
0 -0- 8	0 -1- 7	8 -0- 0	6 -0- 3	4 -2- 4	2 -4- 5
0 -0- 7	0 -2- 8	7 -0- 2	5 -0- 3	3 -0- 2	9 -1- 1
3 -4- 2	4 -0- 5	7 -0- 0	3 -1- 4	1 -0- 9	4 -0- 7
0 -0-10	8 -2- 0	7 -0- 0	10 -0- 0		
0 -0-12	6 -2- 2	6 -0- 4	8 -0- 1		
		10 -0- 0			
10%+	40%+	100%+	90%+	87.5%+	50%+

approximated the mean fertility dose. The results obtained compared favorably with those in the studies with the wheat germ oil concentrate. A daily intake of 0.0375 mgm. sufficed to prevent resorption in 2 of the 5 female rats tested (table 3). The occurrence of dead fetuses in both rats indicates the critical character of this level of dosage which, as will be recalled, did not afford sufficient protection for males. On the other hand, twice this daily intake (0.070 mgm.) proved more than adequate for completion of gestation in females and for maintaining an intact seminiferous epithelium in males. It can be assumed that the minimal protective dose lies between these two levels of intake. Although the data pre-

sented are too limited to permit a conclusive statement concerning the alpha tocopherol requirements of male and female rats, it is apparent that the needs of the two sexes for the synthetic vitamin are very similar. Furthermore, the ratio between the minimal daily dose and the mean fertility dose corresponds closely to that observed in the experiments with wheat germ oil concentrate.

Discussion. Throughout these studies no allowance has been made for possible variations in relationship between the age or the body weight of rats and their vitamin E requirements. The results of bio-assay studies carried out in this laboratory indicate that the reproductive needs of the female are not significantly influenced by differences in body weight. Since the males had attained body weights of 350 to 400 grams by the end of the 90 day experimental period, it does not seem likely that their needs for vitamin E would have increased appreciably had they been continued on experiment for a longer period. It is possible, however, that the lesser effectiveness of the lower level of tocopherol dosage (0.0375 mgm.) in males than in females may be attributed to the relatively larger size of the former sex. Calculated on the basis of the mean body weight of both sexes during the period over which the doses were administered, the males and females fed the fully protective dose of 0.070 mgm. received, respectively, 0.33 and 0.48 mgm. of tocopherol daily, per kilogram of body weight. This represents one-third to one-half the amount of alpha tocopherol, in terms of milligrams per kilogram of body weight, which Mackenzie and McCollum (1940) have estimated to be necessary for protecting rabbits against muscular dystrophy.

Since such very different structures (developing fetus and seminiferous epithelium) are affected by deprivation of vitamin E in the two sexes, it is indeed remarkable that the daily requirements of males and females for the two preparations used have proved so similar. Although we have no information on the tocopherol content of the wheat germ oil concentrate used in these studies, it is generally accepted that wheat germ oils produced in this country contain several times as much alpha tocopherol as beta tocopherol, and probably contain no other biologically active tocopherol. Consequently, the studies presented in this report have been concerned primarily with the minimal requirements of the rat for alpha tocopherol, in its natural and synthetic forms. It is hoped that similar studies may eventually be extended to an analysis of the requirements of male and female rats for the beta and gamma forms of tocopherol, and for chemically related compounds possessing biological activity characteristic of vitamin E.

Our observations confirm those of Evans, Emerson and Emerson (1939) concerning the ability of synthetic alpha tocopherol to function as vitamin E in the male as well as in the pregnant female. On the assumption that the male rat requires considerably more vitamin E than the female,

these investigators fed daily doses of 80 mgm. of wheat germ oil to five male rats and 0.75 mgm. of alpha tocopherol to six other males, otherwise deprived of vitamin E, from the 21st to the 277th day of life. On the basis of their data and our experimental findings, their rats received approximately three and fifteen times the minimal requirement of wheat germ oil and tocopherol, respectively. Although the testes were normal in both groups, the rats fed wheat germ oil showed defective sex interest. This is in keeping with the lower intake of E in this group and suggests that the vitamin requirements for functional normality of the male rat may be somewhat greater than for maintenance of a normal seminiferous epithelium. Unfortunately, no mating tests were carried out on the males of our series. Changes in the interstitial tissue of the testis and in the epithelial lining of the accessory sex glands, which Evans et al. (1939) describe as being particularly marked in their tocopherol fed rats, were not observed in our animals. It seems unlikely that the shorter period of experimental feeding in our studies can account for these differences in observation. It is also of interest that Koneff (1939), reporting on the cytological characteristics of the anterior pituitary glands of the rats used in Evans' study, states that "in the majority of the respects studied there was an improvement toward normality in the following order: E-low → alpha tocopherol → wheat germ oil → natural food." These findings are not in accord with the levels of vitamin E intake in the different groups. Further investigations are necessary before it can be stated with certainty that synthetic alpha tocopherol can completely replace vitamin E from natural sources. The studies reported here indicate that the synthetic and natural forms of tocopherol are about equally effective in permitting normal reproduction in the female rat and in maintaining structural integrity of the reproductive system in the male.

SUMMARY AND CONCLUSIONS

1. Male rats deprived of vitamin E from the beginning of life require a definite source of the vitamin prior to the 40th to 50th days of age in order to prevent irreversible physiological disturbances in the germinal epithelium which antedate, by 10 days or more, the onset of histopathological changes; whereas the reproductive needs of the female rat for vitamin E arise subsequent to conception and implantation, which may occur at any stage of the reproductive period.

2. It is emphasized that these qualitative differences in sex requirements for vitamin E must be taken into consideration in the evaluation of experimental data interpreted as demonstrating the existence of separate anti-sterility factors, or the need of different amounts of a single factor, for the prevention of sterility in male and female rats reared on diets deficient in vitamin E.

3. The daily administration of 2 mgm. of a concentrate from wheat

germ oil fed orally from the 10th day after weaning to rats depleted of vitamin E from early life proved equally effective in preventing sterility in male and female rats, but daily doses of 1 mgm. or less were inadequate.

4. Similar administration of 0.0375 mgm. of synthetic alpha tocopherol caused a slight delay in the onset of testicular degeneration in males and permitted 2 out of 5 females to complete gestation; while twice this dose proved more than adequate for the prevention of sterility in both sexes. The minimal protective dose must lie between these two levels of intake.

5. It is concluded that the minimal daily requirements of vitamin E (as wheat germ oil concentrate or as synthetic alpha tocopherol) for preventing sterility in E-deficient rats are essentially the same for both sexes and, on the basis of bio-assay tests, represent between one-fifteenth and one-twentieth the mean fertility dose for adult female rats.

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ON THE VAGO-INSULIN AND SYMPATHETICO-ADRENAL SYSTEM AND THEIR MUTUAL RELATIONSHIP UNDER CONDITIONS OF CENTRAL EXCITATION INDUCED BY ANOXIA AND CONVULSANT DRUGS[1]

J. FELDMAN, R. CORTELL AND E. GELLHORN

From the Department of Physiology and Psychiatry, College of Medicine, University of Illinois

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McQuarrie, Ziegler, Wangensteen and Dennis (1939) showed recently that whereas anoxia produces hyperglycemia in normal dogs it causes a fall in blood sugar in adrenalectomized animals. In order to explain this interesting phenomenon we have repeated their experiments in rabbits and rats and analysed them further by studying the rôle of the splanchnics and of the vagi. The experiments seem to indicate that under the influence of anoxia both parasympathetic and sympathetic centers are excited. Evidence is presented to show that the secretion of insulin may be stimulated by the action of oxygen lack on autonomic centers.

It seemed to us of interest to supplement these investigations by studying under similar conditions the action of a drug which is known to produce marked sympathetic effects by central stimulation of autonomic centers. Metrazol was chosen because it increases the excitability of sympathetic hypothalamic centers as measured by the height of contraction of the nictitating membrane, thus acting similarly to anoxia (Carlson, Darrow and Gellhorn, 1940). Moreover, Gellhorn and Darrow (1939) showed that intravenous injection of metrazol in convulsive and subconvulsive doses may lead to prolonged discharges from the nictitating membrane (n.m.). The analysis of the pupillary changes showed a marked dilatation due to inhibition of the parasympathetic tone and excitation of the sympathetic; but a temporary phase of pupillary constriction could also be observed in the sympathectomized eye. Metrazol like anoxia causes a sympathetico-adrenal discharge resulting in hyperglycemia. This rise in blood sugar is prevented by splanchnicotomy (Bömer, 1930).

The experiments reported in this paper show that the similarity of

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the action of anoxia and metrazol goes still farther since both factors elicit insulin secretion via the vagi.

METHODS. Anoxia was induced by the inhalation of 7 per cent oxygen for two hours in rats and for three hours in rabbits. No narcosis was employed. Concerning the technic, compare Gellhorn and Packer (1940). The rabbits were first subjected to a typical anoxia experiment. Then the adrenal were denervated bilaterally and the anoxia experiment was repeated after an interval varying from 7 days to 4 weeks.

Forty-nine experiments were performed on rats. These were divided into three groups. The first group comprised the controls which inhaled 7 per cent oxygen for two hours, the second group of experiments was performed on adrenalectomized rats which had been operated on three to seven days earlier and the third group consisted of rats in which not only the adrenals had been removed but which had also been vagotomized below the diaphragm. The blood sugar was determined prior to the experiment and after a period of two hours of anoxia.

A similar group of experiments was performed on rats in which instead of anoxia the effect of 55 mgm. metrazol per kilogram injected subcutaneously was studied. The blood sugar was determined in intervals of 15 and 30 minutes. All animals were starved for 18 hours. The blood sugar was determined by means of the Somogyi modification of the Shaffer-Hartman method. The adrenalectomized animals were allowed to drink a 2 per cent solution of NaCl.

RESULTS. It has been found in numerous experiments that the inhalation of 7 per cent oxygen causes the blood sugar to rise regularly in unanesthetized rabbits (Gellhorn and Packer, 1939). If, however, the adrenal glands were denervated prior to the experiment anoxia no longer caused a rise in blood sugar, but produced uniformly a mild hypoglycemia (fig. 1). Since in these rabbits the secretion of cortin is not interfered with it is obvious that the alteration in response of the blood sugar to anoxia is not due to a deficiency in the cortical adrenal hormone.

After it had been established that the response of the blood sugar to anoxia in animals with denervated adrenals is the same as that found by McQuarrie and collaborators in adrenalectomized dogs, further experiments were conducted on the effect of anoxia on the blood sugar of normal and adrenalectomized rats and of animals in which in addition to the adrenalectomy the vagi had been sectioned intra-abdominally. The results are illustrated in table 1. They show clearly that normal rats respond to anoxia with the usual increase in blood sugar whereas adrenalectomized rats react with a fall in blood sugar, thus confirming the results of McQuarrie in adrenalectomized dogs. The third series of experiments in which in addition to the elimination of the adrenals the vagi had been divided intra-abdominally is particularly interesting.

It was thought that the fundamental difference in the reaction of normal and adrenalectomized rats is due to the fact that in the latter the sympathetico-adrenal system can no longer be stimulated by oxygen lack but this factor may still influence the parasympathetic centers and thereby elicit the secretion of insulin via the vagi. If this hypothesis is correct then it must be assumed that the elimination of the vagi in the adrenalectomized rats would abolish the hypoglycemic effects of anoxia. Table 1 shows indeed that in no instance did a hypoglycemic effect occur in these animals after anoxia. Instead, a slight and statistically insignificant increase of blood sugar is found. The experiments seem to indicate that anoxia stimulates parasympathetic and sympathetic centers at the same time. In the normal animals the stimulation of the sympathetico-adrenal system greatly overbalances the effects

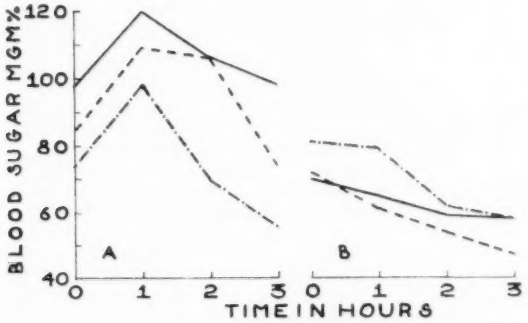

Fig. 1. Effect of inhalation of 7 per cent oxygen on the blood sugar of three rabbits before and after denervation of the adrenals.

on the parasympathetic-insulin system. If, however, the former is removed, the effects on the latter become apparent.

It was of interest to investigate the effect of metrazol on the blood sugar under similar conditions. If metrazol acts on both parasympathetic and sympathetic centers, a reaction similar to that produced by anoxia would call forth a secretion of insulin via the vagi which is normally masked by the more powerful excitation of the sympathetico-adrenal system. Therefore it was to be expected that, whereas metrazol causes a hyperglycemia in normal animals, it would call forth a lowering of the blood sugar in adrenalectomized animals.

Table 2 shows that this is in fact the case. The results are therefore quite comparable to the experiments on anoxia and this holds likewise for the third series of experiments in which the influence of metrazol on the blood sugar was determined in adrenalectomized and vagotomized rats. No statistically significant changes in blood sugar are observed.

The experiments described in this paper show clearly that anoxia and metrazol stimulate the sympathetico-adrenal system to such an

TABLE 1

Effect of 2 hours of anoxia (7% O_2) on normal, adrenalectomized, and adrenalectomized-vagotomized rats

NUMBER	FASTING BLOOD SUGAR	BLOOD SUGAR AFTER 2 HOURS	PER CENT CHANGE
	A. Effect on normal rats		
	mgm. per cent	*mgm. per cent*	
1	70.9	78.5	10.7
2	72.0	83.8	16.4
3	75.2	92.5	23.0
4	73.1	75.2	2.9
5	70.9	86.0	21.2
6	72.0	102.1	41.8
7	68.8	88.2	28.2
8	70.9	86.0	21.2
9	69.8	66.6	−4.6
10	69.8	83.8	20.1
Av	71.3	84.3	18.1
Stand. Dev.	1.75	9.15 $P < 0.01$	
	B. Effect on adrenalectomized rats		
1	63.4	41.9	−33.9
2	65.5	43.0	−34.4
3	64.5	40.8	−36.7
4	63.4	46.2	−27.1
5	62.3	40.8	−34.5
6	58.0	41.0	−29.3
Av	62.8	42.3	−32.6
Stand. Dev.	2.39	1.92 $P < 0.01$	
	C. Effect on adrenalectomized-vagotomized rats		
1	60.2	65.6	9.0
2	58.0	60.2	4.0
3	62.3	70.9	13.8
4	61.2	62.3	1.8
5	58.0	55.9	−3.6
6	60.2	60.2	0.0
7	61.2	64.5	5.4
8	59.1	68.8	16.4
Av	60.0	63.6	5.9
Stand. Dev.	1.49	4.60 $P = 0.04$	

extent in the normal animal that they completely obscure the simultaneous excitation of the vagus which leads to hypoglycemia by means of increased insulin secretion. If this interpretation is correct it should

be possible to show that adrenalectomized animals have more insulin circulating in the blood at the end of the anoxia period than they had

TABLE 2

Effect of metrazol (55 mgm./kgm.) subcutaneously on the blood sugar of normal, adrenalectomized, and adrenalectomized-vagotomized rats

NUMBER	BEFORE METRAZOL	AFTER METRAZOL		
		15 minutes	30 minutes	60 minutes
A. Effect on normal rats				
1	70.9	93.5	96.8	99.9
2	70.9	86.0	72.5	70.9
3	72.0	107.5		
4	70.9	88.2	86.0	79.5
5	73.1	107.5	116.1	156.9
6	69.8	78.5	107.5	86.0
7	70.9	101.0	90.3	82.7
8	72.0	96.8	111.8	107.5
9	70.9	104.2	106.4	96.8
10	69.8	110.0	101.0	82.7
Av.	71.12	97.32	99.01	96.0
Stand. Dev.	1.05	3.18 $P < 0.01$	12.03 $P < 0.01$	23.91 $P < 0.01$
B. Effect on adrenalectomized rats				
1	68.8	43.0	45.1	
2	59.1	51.6	44.0	53.7
3	61.2	50.5	51.6	56.9
4	64.5	50.5	58.0	59.1
5	60.2	52.6		
6	62.3	50.5	51.6	59.1
7	59.1	48.3	46.2	54.8
8	59.1	58.0	49.4	52.6
Av.	61.8	50.62	49.4	56.03
Stand. Dev.	3.19	3.92 $P < 0.01$	4.50 $P < 0.01$	2.53 $P < 0.01$
C. Effect on adrenalectomized-vagotomized rats				
1	58.0	58.0	72.0	64.5
2	59.1	61.2	61.2	60.2
3	58.0	56.9	61.2	59.1
4	63.4	64.5	63.4	
5	58.0	59.1	73.1	56.9
6	56.9	86.0	72.0	58.0
7	61.2	77.4	83.8	70.9
8	58.0	63.4	62.3	59.1
Av.	59.07	65.81	68.62	61.24
Stand. Dev.	2.02	9.77 $P = 0.09$	7.51 $P < 0.01$	4.53 $P = 0.23$

prior to this experiment. The assay of insulin in very small amounts in the blood is of course a very difficult matter. As a method of assay

the effect of the intraperitoneal injection of blood in adrenalectomized mice was used. Brugsch (1930) has shown that the mouse may be used for the assay of small amounts of insulin. We tried to improve the sensitivity of his method by employing adrenalectomized mice instead of normal mice (cf. Hemmingsen and Nielsen, 1938). As table 3 shows it was found that two hours after adrenalectomized mice had been injected with 0.75 cc. of the blood of adrenalectomized rats the blood

TABLE 3

Assay of blood for insulin using the adrenalectomized mouse as a test animal

The values of blood sugar of adrenalectomized mice 2 hours after the intraperitoneal injection of the blood to be tested

Mice injected with blood from normal, adrenalectomized, anoxic and adrenalectomized, anoxic and adrenalectomized-vagotomized, metrazol treated adrenalectomized, and metrazol treated adrenalectomized-vagotomized rats

1 NORMAL RAT	2 ADRENALECTOMIZED RAT	3 ANOXIC AND ADRENALECTOMIZED RAT	4 ANOXIC AND ADRENALECTOMIZED-VAGOTOMIZED RAT	5 METRAZOL* TREATED ADRENALECTOMIZED RAT	6 METRAZOL* TREATED ADRENALECTOMIZED-VAGOTOMIZED RAT
59.1	62.3	49.4	66.6	48.3	65.5
56.9	62.3	53.7	63.4	44.0	60.2
60.2	60.2	52.6	64.5	55.9	59.1
	61.2	53.7	64.5	46.2	64.5
	59.1	52.6	64.5	43.0	68.8
	62.3	54.8	60.2	47.3	64.5
	60.2	55.9			
	58.0	51.6			
	59.1	52.6			
		51.8			
		49.4			
Av. 58.7	60.5	52.6	64.0	47.5	63.8
Stand. Dev.	1.51	1.91	1.93	4.19	3.26
		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P = 0.02$

* Injected with 55 mgm. metrazol/kgm. subcutaneously.

sugar of the mice averaged 60.3 mgm. per cent. If, however, blood was withdrawn from the adrenalectomized rats at the end of an anoxia period the mice tested showed blood sugars averaging 52.6 mgm. per cent. The difference between the two blood sugars corresponds to that produced by about 0.0015 unit of insulin per cubic centimeter. Although this amount is very small, the consistency of the results seems conclusive proof that during anoxia insulin is being secreted by impulses reaching the pancreas via the vagi.

This conclusion is supported by further experiments in which the

blood of adrenalectomized and vagotomized rats was tested for insulin at the end of the anoxia period. As table 3 shows, there was no indication of an increased insulin concentration in the blood but a slight hyperglycemic effect of doubtful physiological significance occurred.

Similar experiments using metrazol were performed on adrenalectomized rats with and without subdiaphragmatic vagotomy. It was found in agreement with the results on anoxia that the insulin content of the blood of adrenalectomized rats was increased after metrazol but such increase was not found after the adrenalectomized animals had been subjected to sectioning of the vagi.

Discussion. The stimulation of autonomic centers by anoxia is probably exclusively due to the action of oxygen lack on chemoreceptors in the sino-aortic area (cf. Gellhorn and Lambert, 1939) whereas metrazol seems to act directly on the autonomic centers since its action is not interfered with by the removal of the buffer nerves (Gellhorn and Darrow, 1939; Carlson, Darrow and Gellhorn, 1940). It seems therefore not improbable that other drugs and physiological factors which are known to stimulate the sympathetico-adrenal system may at the same time excite the vago-insulin system. This seems in fact to be the case. It is well known that insulin hypoglycemia leads to a stimulation of the sympathetico-adrenal system (Cannon, McIver and Bliss, 1924; Schlossberg, Sawyer and Bixby, 1933; Ernstene, Riseman, Stern and Alexander, 1935; Domm and Gellhorn, 1940; Tietz, Dornheggen and Goldman, 1940). Only in conditions of very severe hypoglycemia, signs of vagal excitation become manifest (Himwich and collaborators, 1939). If, however, indicators are chosen which allow one to study increased parasympathetic activity without the interference of increased sympathetico-adrenal excitation results are obtained which seem to permit of a similar interpretation as was given for the experiments on anoxia and metrazol.

Recent observations of Bender and Siegel on the effect of hypoglycemia on the completely denervated iris indicate the liberation of adrenalin and in some cases of acetylcholine in this condition. With the adrenals intact pupillary dilatation (adrenalin) prevails, but after their removal miosis (acetylcholine) may occur. The authors fail to give an adequate explanation of this interesting phenomenon. In the light of the experiments reported in this paper it seems likely that hypoglycemia, similarly to anoxia, leads to the excitation of sympathetic and parasympathetic centers and that the elimination of the more powerful effects of sympathetic stimulation by removal of the adrenals allows one to recognize the simultaneous effects of parasympathetic excitation which lead to the liberation of acetylcholine.

Some observations of Bodo, Cotui and Benaglia which these authors

were unable to interpret may be adequately understood on the same basis. They found that morphine causes a hyperglycemia in normal cats, a slight increase in blood sugar after denervation of the adrenals but a fall in blood sugar in completely sympathectomized cats. If morphine, like metrazol, excites parasympathetic and sympathetic centers the elimination of the sympathetic system must cause hypoglycemia via the vagi.

On the basis of this discussion it is assumed that not only anoxia and metrazol but also hypoglycemia and morphine produce an excitation of both sympathetic and parasympathetic centers. As far as the balance between the two divisions of the autonomic system is concerned it is definitely shifted toward the sympathetic side in the normal animal. If, however, the sympathetico-adrenal system is eliminated, the alteration in the blood sugar level and its dependence on the integrity of the vagi is proof that anoxia and metrazol (and probably hypoglycemia, morphine and other drugs known to stimulate sympathetic centers) act on sympathetic and parasympathetic centers at the same time. The physiological predominance of the sympathetic over the parasympathetic becomes even more marked when the autonomic centers are stimulated. It seems of considerable interest to point out that central autonomic excitation by anoxia, metrazol (and probably morphine and hypoglycemia) involves both divisions of the autonomic nervous system. Obviously the law of reciprocal innervation does not hold for conditions in which autonomic centers are excited under physiological conditions or by means of drugs.

SUMMARY

1. Whereas inhalation of 7 per cent oxygen causes hyperglycemia in normal rabbits it lowers the blood sugar in rabbits with denervated adrenals.

2. Inhalation of 7 per cent oxygen raises the blood sugar in normal rats and causes hypoglycemia in adrenalectomized rats (confirming the work of McQuarrie and collaborators on dogs). If the vagi are divided intra-abdominally in adrenalectomized rats these animals show either no change in blood sugar or a slight hyperglycemia in response to anoxia.

3. Similar experiments were conducted on the effects of metrazol on the blood sugar in rats. Metrazol causes hyperglycemia in normal and hypoglycemia in adrenalectomized rats. After subdiaphragmatic vagotomy adrenalectomized rats respond to metrazol with no change or a slight rise in blood sugar.

4. The results reported under 2 and 3 are interpreted to mean that anoxia and metrazol cause a stimulation of both the sympathetico-adrenal and the vago-insulin system, the effect on the latter being

masked by the more powerful stimulation of the former. This conclusion is substantiated by the assay of blood of adrenalectomized rats for insulin by using the adrenalectomized mouse as a test animal. It was found that blood obtained from adrenalectomized rats at the end of the anoxia period or after injection of metrazol produced a fall in blood sugar in the test animal. Such a hypoglycemic effect was regularly absent when adrenalectomized and vagotomized animals were subjected to anoxia and metrazol respectively and the blood was assayed for insulin.

5. Since, under conditions of stimulation of autonomic centers with metrazol or (reflexly) by anoxia, parasympathetic and sympathetic centers are activated at the same time it does not seem justified to apply the concept of reciprocal innervation to central autonomic processes.

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